

Modelling the impact of stressors on the honeybee colony

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Abstract

The Western Honeybee (*Apis mellifera*) is an important species, not only ecologically and economically, but as a source of recreation to many. The pollination services the species provides benefit a number of crops worldwide, and, as the honeybee is domesticated and kept in hives, can be directed commercially. Recently, although overall global stocks are growing, there have been reports of high colony losses worldwide. Due to the value of this species, this is a worrying trend. There are many stressors facing the honeybee, both natural and anthropogenic in origin. Two of the most prevalent, both in the popular media and in monitoring studies of colonies are insecticidal pesticides and the parasitic mite *Varroa destructor*. Due to the difficulties and expense of carrying out large-scale field studies required to properly investigate the multiple stressors and their interaction, the use of modelling to explore the problem and direct field work is a vital resource.

In this thesis, I present research using the BEEHAVE model and a novel model to explore the exposure and potential impacts of pesticides and the varroa mite. The results show that the timing of a pesticide exposure in the year greatly changes the resultant impact on the colony. Pesticides can have many impacts on different stages of the honeybee, and I show that increased mortalities of different life stages of the honeybee (larvae, in-hive adults, foragers) and decreasing egg-laying rate, affect the development of the colony to different extents at different times of the year, with the colony being highly sensitive to losses of in-hive bees during the summer, and the over-wintering bees at the beginning and end of the year. A novel model is

presented exploring the in-hive distribution of pesticide-containing nectar and the effect it has on the exposure of in-hive receiving bees and larvae. The results from this model show that, in-hive distribution is not important to consider for the adults, but may be for the larvae. The landscape, specifically the distance to pesticide-treated forage in relation to untreated forage also has an impact on the result of a pesticide exposure, and this is a potential avenue for the mitigation of pesticide impacts. I also present work towards the validation of BEEHAVE with regards to varroa mite infestation, finding that the model results are close to empirical data, both for datasets from the UK and USA, but the impact of varroa is underestimated.

The results are discussed in the context of pesticide risk assessment, the mitigation of potential stressors and the modelling of the varroa mite. The BEEHAVE model is a vital tool for many applications, one being the risk assessment of pesticides. A review of the model by the European Food Security Agency (EFSA) highlighted extensions to the model required before it can be incorporated. This research begins to answer some questions asked in that review.

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I love deadlines. I love the whooshing noise they make as they go by

- Douglas Adams, The Salmon of Doubt

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Chapter 1 – Introduction

1.1 Aims

The overarching aim of my research is to use ecological modelling to establish how pesticides and other stressors can impact the honeybee colony. I have done this by a combination of large-scale simulations using the BEEHAVE model (Becher *et al.*, 2014), with alterations to simulate the impact of pesticides, as well as some validation and calibration of the BEEHAVE model with regards to the impact of varroa mites (*Varroa destructor* (Anderson and Trueman, 2000)) on the modelled colony. I have also produced a novel model to explore the potential impacts of in-hive pesticide distribution in the foraged nectar (specifically to establish the complexity required in a model to establish a conservative estimate of the exposure of in-hive bees (adults and brood) to pesticides,

To understand the framing of my research questions, I will first introduce the honeybee, its biology, foraging ecology, and discuss evidence of increased colony losses. I will then present the major stressors to the honeybee colony. As my research is focused on the modelling of the honeybee, I will briefly introduce the concept of ecological modelling, and the place of modelling in understanding how these stressors can impact the honeybee.

1.2 The Honeybee

The Western honeybee (*Apis mellifera*, hereafter honeybee) is a widely-distributed eusocial insect from the Order Hymenoptera. This species is largely domesticated, and kept recreationally and commercially for honey production and pollination services (Crane, 2013).

1.2 Pollination and the importance of the honeybee

Angiosperms are highly reliant on animal pollination, with ~88% of species being pollinated by animal species as opposed to abiotic pollination, such as via wind (Ollerton, Winfree and Tarrant, 2011). Honeybees are important both environmentally and economically as they act as important pollinators for both wild flowers and agricultural crops (Klein *et al.*, 2007; Gallai *et al.*, 2009; Breeze *et al.*, 2011; Gaines-Day and Gratton, 2016). 35% of the world's crops rely on animal pollination (Klein *et al.*, 2007) to survive, and many crops benefit from pollination (Klein *et al.*, 2007). In 2005 it was estimated that pollinated crops were worth 153 billion Euros (Gallai *et al.*, 2009), making pollination of great economic importance, on top of the obvious ecological benefit. Honey is also an important product from the honeybees, estimated to be worth around \$1.25 billion in 2007 (vanEngelsdorp and Meixner, 2010). The extent to which the honeybee itself is responsible for pollination has been scrutinised, with recent estimates placing the actual contribution of honeybees to pollination services at between 11.7% at the least favourable and 34% at the most (Breeze *et al.*, 2011), in either case, a large proportion attributable to a single species. Important to note is that, as the species is domesticated, it is possible to some extent for farmers

to direct pollination to the required crops using honeybee hives, which is not so feasible with wild bees.

1.3 Honeybee biology and ecology

To be able to accurately model a species, having a good understanding of its biology is crucial, if the model is to reliably predict reality.

1.3.1 Honeybee Biology

The honeybee is a highly social insect, living in colonies reaching tens of thousands of individuals. The colonies consist of female workers, a female egg-laying queen and male drones, the latter of which contribute little to the colony outside of fertilisation of new queens. Within the colony, the workers construct combs of hexagonal cells from wax, where the queen lays eggs and workers store pollen and nectar. Worker bees display age polytheism, their role within the hive changing as they age (Robinson, 1987; Winston, Mark, 1991) with young bees performing duties inside the hive, such as brood care, maintenance and queen tending, then moving to guard duty and finally foraging, which they carry out until death.

Eggs are laid in the comb cells by the queen, and hatch after around 3 days. The eggs hatch into larvae and are fed by adult workers for around 6 days. For the first 3 days, all workers are fed 'royal jelly', secretions from the adult worker's hypopharyngeal glands. After this, worker larvae are fed a mixture of honey and pollen (providing carbohydrates and protein respectively), and the queen larvae are continually fed the royal jelly, promoting the required growth to become new queens. The worker bees

cover larval cells containing those larvae in the last larval stage with a wax cap. Then, within the cell, the larva spins a cocoon and pupates, metamorphosing into the adult worker. When the adult is fully developed, the pupa ecloses and the adult emerges from the cell as a new worker bee. As the workers age, they undergo a number of physiological changes, which better suit the tasks they carry out at the specific points in their life-cycle (for example: Knecht & Kaatz, 1990).

1.3.2.Honeybee Foraging Ecology

The acquisition of resources from the landscape is an intricate process consisting of several feedback loops and regulatory mechanisms (Seeley, 1995). Nectar and pollen are collected by foragers and the movement of these products from foragers, via the nurse bees to the larvae is a potential route of exposure of the in-hive bees and larvae to pesticides. I explore this route in a novel model in chapter 3. The undisturbed honeybee colony consumes approximately 20kg of pollen and 60 kg of honey each year (Seeley, 1995). When a foraging bee returns to the hive having successfully collected a nectar load from a food patch, they are able to relay information about the location of the food patch, relative to the location of the sun and the distance between the food patch and the colony via a 'waggle dance' (Von Frisch, 1967), sensed in the darkness by other prospective foragers and accompanied by chemical signals (Thom *et al.*, 2007). The waggle dance consists of the bee moving in a figure-8 pattern, consisting of a straight run circling back to the beginning, walking straight again and then circling the other way. The bee 'waggles' as it walks the straight section, with the vigour and length of the wagging indicating distance to the food source. The bee is dancing on a vertical comb and the angle between the perpendicular and the bee's

body when running the straight section of the dance corresponds to the direction of the food in relation to the solar azimuth (point on the horizon directly under the sun's position in the sky). These dances serve to recruit more foragers to the profitable food patches. This process is further regulated by the presence and behaviour of receiver bees, younger bees who meet foragers, receive the nectar and then store the nectar in cells (Anderson and Ratnieks, 1999). If a forager is required to wait for a receiver bee for a period of time, implying that the influx of nectar is high, it may perform a tremble dance, promoting the recruitment of other in-hive bees to work in nectar processing, while also informing other foragers to not recruit to their nectar patch (Seeley, 1992). Through the interpretation of queuing delays (Ratnieks and Anderson, 1999; Thenius, Schmickl and Crailsheim, 2008), or through transferring their nectar load to multiple receivers (Hart and Ratnieks, 2001), the foraging honeybee is able to get information about influx of nectar from other sources. The foraging and tremble dances serve to broadly regulate foraging and receiving (Anderson and Ratnieks, 1999), enabling multiple individuals to exploit a number of food sources as efficiently as possible. Due to the intricacies of the regulation of foraging, based on perceived energetic efficiency of food sources, the honeybee colony is highly sensitive to changes in the landscape. The nectar and pollen are used as food, both by the adult bees and as food for the brood. The majority of the pollen collected throughout the year is consumed during the year, however nectar, if there is a large influx will be collected and concentrated by the in-hive bees and stored in the cells as honey. This is capped and provides a food source for later in the year and over-winter when there is little nectar coming into the colony.

As well as foraging for food, the bees will also forage for water and resin. Water is used in thermoregulation on hot days as well as for dilution of the honey when feeding the brood. The regulation of water foraging is similar to that of nectar foraging, but whereas nectar processing is regulated through the change in supply, water foraging is regulated through changes in the in-hive demand (Seeley, 1995), as nectar availability changes throughout the year, whilst water remains mostly constant in the environment. A small number of bees will collect resin from trees in the landscape, used in the building and maintenance of the hive, which may also have antimicrobial properties.

In Chapter 4 of this thesis, I use the BEEHAVE model to assess how changes to the foraging landscape (both a simple and more complex landscape) affect the colony, while also adding a pesticide-induced stress to the foragers, to see how the landscape interacts with the impact of this stress.

1.4 Honeybee population decline

Honeybee populations in many parts of the world have been suffering from an increase in colony losses (Biesmeijer *et al.*, 2006; Neumann and Carreck, 2010; Potts *et al.*, 2010; vanEngelsdorp and Meixner, 2010; vanEngelsdorp *et al.*, 2012; Lee *et al.*, 2015). Periods of increased honeybee colony deaths are not a new phenomenon (Oldroyd, 2007; vanEngelsdorp and Meixner, 2010), but are concerning, considering the honeybee's input into the agricultural ecosystem. The overall global stock of managed honeybees is increasing (Aizen and Harder, 2009; Potts *et al.*, 2016), perhaps due to the action of beekeepers, as are honeybee stocks in the USA (from

2.39 million colonies in 2006 to 2.64 million colonies in 2013, Lee *et al.*, 2015), which is promising, but the global honeybee stocks may not be increasing fast enough to meet the increase in demand for insect pollination (Aizen and Harder, 2009). This is made more concerning by the fact that it is not just the honeybee that is seeing population decline or increased colony deaths, but pollinators in general that have been experiencing population losses and decline in diversity (Carvalho *et al.*, 2013; Vanbergen *et al.*, 2014). The importance of insect pollination to agriculture makes the inquiry into the drivers of these population declines and also into how best to reduce or counter them, of the utmost importance, as there is evidence that the decline in species richness may be slowing or even reversing in some countries (Carvalho *et al.*, 2013) although it is unclear on the drivers of this phenomenon.

The United States has experienced large scale colony losses characterised by a set of specific symptoms, referred to as Colony Collapse Disorder (Vanengelsdorp *et al.*, 2009). These symptoms are i) loss of adult bees with brood remaining; ii) a lack of dead adult bees in or around the colony; iii) lack of pests and kleptoparasitism. CCD is likely the result of a number of stressors acting together, although it is important to note that not all colony deaths are as a result of CCD (Ratnieks and Carreck, 2010), however as the disorder is often politicised (Watson and Stallins, 2016), it can be difficult to discern the truth.

1.5 Multiple stressors

The honeybee faces a number of stressors in the environment and in the hives, both as a direct result of human activity or from biotic factors, which themselves may be exacerbated by human activity. Importantly, each stressor may interact with each other stressor forming an intricate stress-landscape. These stressors include: the loss of habitat and forage in the landscape from anthropogenic landscape change (Naug, 2009; Kovacs-Hostyanszki, Batary and Baldi, 2011; Jonsson *et al.*, 2012; Clermont *et al.*, 2015), the use of pesticides on crops and other plants (F Sanchez-Bayo and Goka, 2014; Carreck and Ratnieks, 2015; Godfray *et al.*, 2015; Johnson, 2015), parasites such as *Nosema sp* (Martín-Hernández *et al.*, 2007; Higes *et al.*, 2008; Williams *et al.*, 2014) and *Varroa destructor* (Rosenkranz, Aumeier and Ziegelmann, 2010; Akyol and Yeninar, 2011; Annoscia, Del Piccolo and Nazzi, 2012; Francis, Nielsen and Kryger, 2013) and a number of viral and bacterial diseases (Allen and Ball, 1996; Chen *et al.*, 2006; Highfield *et al.*, 2009; Wilfert *et al.*, 2016).

I will now explore a number of these stressors in more detail to emphasise the importance of their consideration in the realistic modelling of the honeybee colony.

1.5.1 Land use

Human activity is changing the landscape, the requirements of industry and agriculture, amongst other endeavours, have led to a vast conversion of the natural landscape worldwide (Hoekstra *et al.*, 2004). Naug (Naug, 2009) reports a significant prediction of the colony loss in each state by the ratio of open land to developed land. A similar correlation is shown by Clermont *et al.* (Clermont *et al.*, 2015), who find that

industrial, transport or recreational-activity based land cover correlates with increased levels of colony death, and Kovács-Hostyánszki et al. (Kovacs-Hostyanszki, Batary and Baldi, 2011) show that the intensification of farmland (specifically measuring increase in fertiliser and insecticide use) reduce bee abundance and richness, regardless of the percentage of semi-natural habitats nearby, although there is evidence that the use of organic farming methods may not have much beneficial impact (Brittain *et al.*, 2010). It is important to note that these studies are purely correlative, and so imply a link between increased agricultural intensification and colony loss, but give no mechanistic evidence. For the honeybees, the key aspects of importance in the landscape are the availability of pollen and nectar. In 'natural' or semi-natural landscapes there is likely to be a larger variety of plant species, and therefore a larger variety in flowering times, providing a flow of food throughout large parts of the year. As land is used for agriculture, there is an increase in monoculture, this form of agriculture reducing the variety in the timing of forage availability, as for the crops that do provide nectar and pollen, each is likely to produce masses of flowers for a short-lived mass blooming period. During this mass flowering, there will be a large influx of nectar and pollen into the colony, however, outside of this window, there will be a much reduced volume of nectar and pollen available for the colony. The correlation between bee abundance and fertiliser use (Kovacs-Hostyanszki, Batary and Baldi, 2011) is likely due to fertiliser promoting non-flowering grass growth, further reducing floral abundance. Changes to land use are very difficult to reverse, and are something that beekeeper intervention can do little to help with, and, as such, the resultant stresses to the honeybees are important to consider when trying to increase colony health. The use of mechanistic models such as BEEHAVE, which include the

landscape can be very useful in determining how the landscape affects the colony dynamics and how the quality and organization of the landscape interacts with other stressors.

1.5.2 Varroa destructor

1.5.2.1 Biology of *Varroa destructor*

Varroa destructor (Anderson and Trueman, 2000) is a haemophagous mite which reproduces and grows in the capped brood cells of the honeybee (Rosenkranz, Aumeier and Ziegelmann, 2010), and spends some of its adult life attached to the adult bees (preferably a mid-aged nurse bee), feeding from their haemolymph through their cuticle, a period known as the 'phoretic' phase. Originally, it was thought that the mite found in the colonies of the Western honeybee (*Apis mellifera*) were the same as first described in the Eastern honeybee (*Apis cerana*) colonies, *Varroa jacobsoni*, however Anderson and Trueman (Anderson and Trueman, 2000) found that, in fact, the mite found in the Western honeybee was a separate species, *Varroa destructor*.

The mite expresses arrhenotokous haplodiploidy, with unfertilised eggs producing males. Before a larval cell is capped by the nurse bees a female mite enters the brood cell and moves down into the larval food, possibly to avoid detection (Rosenkranz, Aumeier and Ziegelmann, 2010). From here she lays a number of eggs, the first being a male, who is able to fertilise the original female, leading to subsequent eggs producing the larger female mites, the male begins copulating with these females almost as soon as the females appear. When the developing bee emerges from the cell, the mites that entered the cell before it was capped or those that hatched within

the cell emerge and are able to enter the phoretic phase by attaching themselves to the adult bees.

1.5.2.2 How the varroa mite harms the honeybee

Rosenkranz et al. call the varroa mite “the greatest threat to apiculture” (Rosenkranz, Aumeier and Ziegelmann, 2010) and the varroa mite is one of the most important factors in the decline of the honeybee populations (Genersch *et al.*, 2006; Boecking and Genersch, 2008; Dahle, 2010). The mites feed on the haemolymph of the bees, both during the reproductive phase, feeding on the pupa while it is developing, and on the host adult during the phoretic phase. Individuals from mite infested cells show a lower body weight than mite-free bees (Duay, De Jong and Engels, 2003; Annoscia, Del Piccolo and Nazzi, 2012). They may also have less well-developed organs (Schneider *et al.*, 2012). Critically, the Varroa mite acts as a vector for many honeybee viral diseases, as mites infected with a disease will pass the virus to the host bee as the mite feeds on the haemolymph (Bowen-Walker, Martin and Gunn, 1999; Shen *et al.*, 2005; Wilfert *et al.*, 2016). In Hawaii, for example, the presence of the mite increased the prevalence of deformed wing virus (DWV) from ~10% to 100% (Martin et al., 2012). If there are multiple foundress mites infesting the cell of a developing bee, the DWV level in the resultant bee is higher than those developing in cells with a single foundress mite (Khongphinitbunjong *et al.*, 2015), implying that more mites in the hive will lead to a greater transmission of viruses.

Infestation with the varroa mite also affects food gathering as foragers from an infested colony spend longer outside the hive and are less likely to return than foragers from

an uninfested hive (Kralj and Fuchs, 2006)(Kralj *et al.*, 2007), and also suffer from affected learning (Kralj *et al.*, 2007), two sublethal impacts that can damage the host honeybee's colony development. As varroa are so impactful to the honeybee colony (Boecking and Genersch, 2008), a model of the honeybee colony incorporating multiple stressors needs to reliably model the impact and dynamics of any mite populations infesting the colony. In chapter 5, I use two empirical datasets from very different climates to work towards the validation of the BEEHAVE model with regards to the varroa mite.

1.5.3 Viruses

There are 24 viruses associated with the honeybee (McMenamin and Genersch, 2015), many of which impair the honeybee, either physically or cognitively or lead to death of the infected bee (McMenamin and Genersch, 2015). Highfield *et al.* (Highfield *et al.*, 2009) found that DWV loads were significantly related with over winter colony loss in the hives tested, and the same relationship was found by Dainat *et al.* (2012). This is especially worrying as DWV is found globally(Allen and Ball, 1996; Wilfert *et al.*, 2016). A large scale monitoring project in Germany also found that varroa infestation, DWV and acute bee paralysis virus(ABPV) loads, along with queen age, were all significant predictors of colony loss (Genersch *et al.*, 2010). Honeybee viruses have been found in non-apis bees(Genersch *et al.*, 2006) and may pass between the wild non-apis bees and managed honeybees (Fürst *et al.*, 2014).

1.5.3 Nosema

The microsporidian gut parasite *Nosema ceranae* is another major potential factor in the decline of honeybee populations. It was first thought that *Nosema apis* was the major pathogen to *A. mellifera* and that *N. ceranae* was mainly a pathogen to *A. cerana*, hence the name. It would seem, however, that *N. ceranae* has also been a major pathogen to *A. mellifera* for longer than expected (Chen *et al.*, 2008) and the combined effects of *N. apis* and *N. ceranae* could well be major cause of the population decline. Nosema infected foragers have a longer forage time and a lower return rate than 'healthy' foragers. Kralj and Fuchs (Kralj and Fuchs, 2010) believe that this (along with the same effect they found in Varroa (Kralj and Fuchs, 2006), could be an adaption of the honeybees to the infection to lower overall infection in the hive. Williams *et al.* (Williams *et al.*, 2011) however found no significant difference in colony strength or winter mortality between Nosema-positive hives and those treated with the antibiotic 'Fumagilin-B®' even though the antibiotic significantly reduced Nosema spores in the hives. Nosema infestations reduce the effectiveness of the Apistan strips for Varroa control, possibly due to the behavioural effects of Nosema reducing the interactions required for Apistan to work (Botias *et al.* 2012). Pettis *et al.* exposed colonies to imidacloprid levels lower than those shown to cause adverse effects to foraging or longevity and then exposed the newly emerged bees to Nosema. The Nosema infections were higher in the pesticide affected hives than in those not treated.

1.5.4 Pesticides

The honeybee is susceptible to impacts from a number of pesticides. Depending on the pesticide and level of exposure, there are a number of ways these impacts can manifest. I will discuss the most common classes of insecticide used in the UK, then I will discuss the abundance of pesticides in the environment and the resultant exposure of the bees to these pesticides. Finally I will introduce the impacts that these chemicals can have on both the individual bees and the colony as a whole.

1.5.4.1 Pesticide usage in UK

In 2014, counting repeated treatments separately, 6.5 million ha of land in the UK was treated with insecticide. Of this, 66% was with pyrethroid insecticides (the most commonly applied being λ -cyhalothrin and deltamethrin) and 36% was with neonicotinoid insecticides (the most commonly applied being clothianidin) (according to data from Fera's PUS STATS (<https://secure.fera.defra.gov.uk/pusstats/index.cfm>)).

1.5.4.2 Types of insecticide

There are a number of groups of insecticides, all of which act in different ways. At the time of writing, The Insecticide Resistance Action Committee list 27 insecticidal modes of action (Sparks & Nauen 2015 list 25, since then two more have been added).

Pyrethroids – impair the action of the voltage-gated sodium channels in the axial membranes of the neurons. This stops the nerve from carrying action potentials, leading to paralysis.

Neonicotinoids – bind to the nicotinic acetylcholine (nACh) receptors in the post synaptic membrane. This stops the neurotransmitter from binding itself and leaves the synapse unable to carry the signal. These are specific to insect nACh receptor.

Carbamates – Inhibit the enzyme acetylcholine esterase, the enzyme that breaks down the acetylcholine in the synaptic cleft. Without this enzyme, the acetylcholine continues to bind to the receptors in the post-synaptic membrane, continuously causing a signal in the post-synaptic neuron. This inhibition is temporary and will reverse.

Organophosphates – Inhibit the enzyme acetylcholine esterase, as with the carbamates, however the inhibition is irreversible.

When considering the movement of pesticides in the landscape in the context of potential exposure to the honeybee, one vital feature of the chemical in question is whether it is delivered systemically. A systemic pesticide is a pesticide that, usually due to a higher solubility in water, will be taken up into the plant from the soil. For example, Clothianidin is a systemic neonicotinoid insecticide, has a solubility in water of 327 mg/L (at 20°C), deltamethrin, a non-systemic pyrethroid has a solubility in water of between 0.002-0.0002 mg/L. This uptake into the plant can serve to provide resistance to the plant without the need for foliar sprays.

1.5.4.3 Application types

Insecticides are applied to plants in three main ways:

1) Foliar spray – the chemical is applied via a spray onto the plant. This can occur at various scales, from large-scale aerial spraying to small-scale spraying by hand. Depending on weather conditions and application methods, this may lead to pesticide drifting onto non-target plants (Felsot *et al.*, 2010).

2) Seed dressing – The pesticide is coated onto the seed of the crop before it is planted. For insecticides, this is commonly a systemic insecticide, which will be taken up by the developing plant and provide long-term protection throughout the plant's development, removing the need for spraying in theory.

3) Granular application – The pesticide is applied to the soil as a granule, which will spread the chemical into the soil.

A pesticide is typically not applied as a single active ingredient. Instead, sprays and seed treatments are applied as a formulation with one or more pesticides, (for example, multiple insecticides or an insecticide and a fungicide) with a number of adjuvants. These include chemicals acting as solvents or surfactants and while they are in general inert, they can affect the formulation's behaviour in the environment and it has been shown that the formulation can affect the impact on an individual organism (Mullin *et al.*, 2015). Finally, the metabolites or breakdown products of a pesticide may also have harmful effects, for example, the neonicotinoid thiamethoxam breaks down into the neonicotinoid clothianidin.

1.5.4.4 Movement of pesticide into plants

Regardless of the application method, there is likely to be pesticide on or within the soil. The movement of pesticides within the soil is a complex process, depending on the chemistry of the pesticide (K_{ow} , solubility in water), formulation applied (e.g. proportion of solvent or surfactant), climatic conditions (e.g. temperature and rainfall) and features of the soil, including the microbial communities present (Gavrilescu, 2005; Bansal, 2011). If there is pesticide around the roots of the crop, then, again dependent on the chemistry of the pesticide (Briggs, Bromilow and Evans, 1982) and the concentration of the pesticide around the root, then the plant may take up the pesticide. Once the pesticide has been taken up by the roots, if it is a highly soluble chemical, it may be carried within the plant and be distributed to the nectar, pollen (Barker, Lehner and Kunzmann, 1980; Stoner and Eitzer, 2012) and guttation fluid of the plant (Girolami *et al.*, 2009).

1.5.4.5 Movement of pesticide into the hive

As the foraging honeybees visit plants that either have been, or are being, sprayed with a pesticide or a plant that has taken up pesticide from the soil, they may be exposed to the pesticide. There are two main routes of exposure. The first is *contact* (or dermal) exposure. This is when the insect contacts the pesticide with its cuticle, This could be direct spray or as a result of spray drift (Longley *et al.*, 1997) or from landing on a plant that has been sprayed with a pesticide recently enough to have retained a pesticide residue on its surface. In Italy in 2000 (Schnier *et al.*, 2003) and Germany in 2008 (Pistorius *et al.*, 2010) there were incidents of the dust from planting pesticide-coated seeds being carried by the wind exposing the bees to the pesticide

in high doses, which could be exposure through contact, however with the proper drilling equipment, this is reduced (Nikolakis *et al.*, 2010). The second route of exposure is *oral* exposure. This occurs when an individual consumes a pesticide, through contaminated nectar (or possibly honey for in-hive bees or brood), pollen or water (Girolami *et al.*, 2009; Samson-Robert *et al.*, 2014).

1.5.4.6 Movement and presence of pesticide within the hive

Once pesticides enter the hive in products carried by the foragers, they will be distributed within the hive ecosystem. Water is used to dilute the stored honey and in temperature regulation. The pollen and nectar, however, are stored in the cells of the comb to be used as food or stored for later use. This leaves a number of compartments in which pesticide can be found: the bees themselves, honey (or nectar), pollen and the wax from which the comb is made.

A number of pesticides are applied directly to the hive to treat for the varroa mite. Historically these include coumaphos (an organophosphate) and tau-fluvalinate (a pyrethroid). Studies examining pesticide residues find that most, if not all samples of the wax contain these two chemicals, as does the pollen (Chauzat *et al.*, 2009; Mullin *et al.*, 2010; Bonzini *et al.*, 2011; Wu, Anelli and Sheppard, 2011), whereas, the nectar or honey, has a higher percentage of samples containing neonicotinoids (Chauzat *et al.*, 2009; Pohorecka *et al.*, 2012). Tremolada *et al.* (Tremolada *et al.*, 2004), find that the partition coefficient between honey and wax is similar to that between octanol and water. This could explain the high movement of coumaphos into the wax (Tremolada *et al.*, 2004), and why the less lipophilic neonicotinoids are found more in the honey.

1.5.4.7 Impacts of pesticides on the honeybee

A pesticide can have a number of impacts on an individual honeybee. The impact will depend on the pesticide in question as well as the level of exposure. A pesticide may kill the individual, a *lethal* effect. The death from the pesticide could be immediately, an *acute* lethal effect or some time after exposure, a *chronic* lethal effect. A lethal effect is commonly measured as an LD₅₀ or LC₅₀, or some variant. This is the dose (LD₅₀) or concentration (LC₅₀) of pesticide required to, statistically, kill 50% of a test population. These can either be acute or chronic, measured soon after exposure or some time after exposure. Many insecticides can have a lethal impact to the individual honeybee (Bailey *et al.*, 2005).

In many cases, if the exposure to the pesticide was lower than that required to kill, it will still have some effect on the individual, so called *sublethal* effects (Desneux, Decourtye and Delpuech, 2007; Thompson and Maus, 2007; Aliouane *et al.*, 2009; Vidau *et al.*, 2011; Belzunces, Tchamitchian and Brunet, 2012; Williamson, Baker and Wright, 2013; Charpentier *et al.*, 2014; Wu-Smart *et al.*, 2016). These sublethal effects can be *behavioural*, leading to a change in the behaviour of the individual, or *physiological*, causing some sort of change to the individual. There are many different sublethal effects that have been reported from pesticides onto the honeybee. They include: reduced memory (Decourtye, Lacassie and Pham-Delègue, 2003; Ramirez-Romero, Chaufaux and Pham-Delegue, 2005; Aliouane *et al.*, 2009; Abramson *et al.*, 2012; Williamson and Wright, 2013; Williamson, Baker and Wright, 2013), reduced foraging and feeding (Nauen, Ebbinghaus-Kintscher and Schmuck, 2001; Ramirez-Romero, Chaufaux and Pham-Delegue, 2005; Schneider *et al.*, 2012; Tan *et al.*,

2014), and impaired colony reproduction and development of workers, drones and queens (Bendahou, Bounias and Fleche, 1999; Haarmann *et al.*, 2002; Pettis *et al.*, 2004; Sharma and Abrol, 2005; Wu, Anelli and Sheppard, 2011; Williamson and Wright, 2013).

A large number of the studies on the effects of pesticides on the honeybee are laboratory studies (Cresswell, 2011; Godfray *et al.*, 2014, 2015), the first tier suggested in the guidance from the European Food Security Agency((Efsa), 2013a). Laboratory studies are often faster and cheaper than semi-field or field studies, and provide a cost-effective means to guide the more complex and costly studies to those chemicals that require them. It is important to acknowledge that the ecologically relevant unit is the colony 'superorganism', and not the individual honeybee. Therefore it is also important to consider the higher tier field studies applying pesticide to a number of colonies to measure the colony-level effects of the pesticide, either with a known dose (or concentration) of the pesticide (either the active ingredient or as a formulation) presented in a nectar feeder or pollen, or with a crop treated with the pesticide at a known application rate. Foraging can be controlled, either by leaving a buffer around the colony with little alternative forage, or by keeping the colony and foraging site in a cage or closed polytunnel to avoid foraging on unknown sources. There have been a number of such tests. Sandrock *et al.* (C. Sandrock *et al.*, 2014) present neonicotinoid to the colony via a pollen patty, finding no increase in over-winter losses, but found short term impacts on colony performance. Tremolada *et al.* (Tremolada *et al.*, 2010) measured the impact of sowing a neonicotinoid treated corn field, finding that hives close to the field suffered an increase in bee mortality and a

reduction in foraging. Rundlöf et al. (Rundlöf *et al.*, 2015), Pilling et al. (Pilling *et al.*, 2013), Cutler et al. (Cutler *et al.*, 2014) and Rolke et al. (Rolke *et al.*, 2016) all examine the impact of a neonicotinoid in oilseed rape (and maize in Pilling et al.) (Pilling et al. - Thiamethoxam, Rundlöf et al., Cutler et al. and Rolke et al. – Clothianidin) and all three large-scale field studies found little impact of these pesticides on the honeybee colonies. These findings imply that, while many insecticides can have many individual-level impacts on the individual honeybee, these may not always translate up to the colony level, but this appears to depend on the crop. In this thesis I will be addressing the impacts of pesticides on the honeybee colony in a number of ways, looking at both the hazard and exposure, or pesticide risk. In chapter 2 I explore how increased individual-level mortality of three of the life stages of the honeybee (larvae, in-hive workers and foragers) scales up to colony level impacts, in an effort to determine which life-stages the colony is most sensitive to losing. In chapter 3 I present a novel model exploring how in-hive pesticide distribution could affect the exposure of in-hive bees to pesticides. In chapter 4 I explore how the quality (in terms of forage availability) of the landscape and a pesticide-induced foraging mortality can interact and impact the colony.

1.6 Ecological modelling

1.6.1 Introduction to modelling

Having covered the biology of the honeybee and the stressors that it faces, I will now introduce the concept of a model, and introduce previous work that has been

performed using modelling to explore the honeybee colony. Through modelling, we are able to perform experiments that would take a lot of time and money to perform empirically. Although the reliability of the predictions from modelling requires a strong empirical base when designing the model, many scenarios can be explored to further understand the system and can this can complement further empirical work.

A model of a system is, essentially, a simplified representation of that system (Grimm and Railsback, 2005), regardless of the nature of the system in question, be it physical, ecological or sociological in nature. A model becomes of explicit use in scientific investigation when it is developed with a specific problem in mind that we seek to answer, whether that problem is the understanding of a system or the prediction of a systems behaviour (Caswell, 1976). As discussed in Grimm and Railsback (Grimm and Railsback, 2005), ecological systems are often very complex, consisting of many individuals, with many traits interacting in many ways. As such, when developing models to answer ecological questions it is necessary to simplify the system. Often this simplification is explicitly required as it is impossible to gather data on or, in many cases, to even quantify these interactions or ecological processes.

1.6.2 Types of models

When modelling an ecological system, there are a number of options for the type of model to use (Jørgensen and Bendoricchio, 2001). There are a number of model types and methods, and the best option is dependent on the features of the system in question and the quality of the data available.

Typically, an ecological model will be modelling the change in a system over time, leading to a *dynamic system*. Whether time is modelled as discrete or continuous will decide whether the equations in the model are difference equations or differential equations, respectively. These models can be deterministic or stochastic, depending on whether the parameters are set as definite values or as probability distributions. In addition, models may require a spatial element, as the landscape in which the population or community exists may not be homogeneous. This type of model can be of a single species, as in the logistic model, or multiple species interacting, as in the Lotka-Volterra equations (Lotka, 1925; Volterra, 1926).

These models assume that every individual in the population is equivalent. In reality, however, individuals will differ in their reproduction and mortality and other functions. This is typically modelled with matrix equations (Caswell, 2001), such as with the *Leslie matrix* (Leslie, 1945) for age-structured populations, applying a different reproduction and mortality rate for individuals of each age, or the *Lefkovitch matrix* (Lefkovitch, 1965) for stage-structured populations, with a different reproduction and mortality rate for each stage (for example, larvae – pupae - adult for an holometabolous insect population) as well as the proportion of each stage moving to the other stages at each time-step.

Finally, the last option is to model the processes and behaviour of the individuals in the system, with the overall behaviour of the system emerging from this. For population modelling, this would be individuals within the population, with the individual biological processes, interactions between individuals and the environment explicitly modelled

and the dynamics of the total population are emergent. This approach is referred to as Individual Based Modelling (IBM) or Agent Based Modelling (ABM) (Grimm and Railsback, 2005). This approach is especially useful in ecology, as for ecological systems, the individuals are not all the same, individuals will adapt in search of maximal fitness within their environment. The use of individual based models captures these local interactions, as opposed to focussing on global changes, and by their nature can incorporate differences between individuals by stage, age or at a higher resolution if necessary. As it is the individual that is being modelled, spatial features can also be incorporated in the model as the position of each individual in the landscape is calculated. Classical mathematical models suffer from an increase in the difficulty of analysis as complexity increases. IBMs, are able to incorporate more complexity and 'realistic' procedures without sacrificing the ability to analyse the results.

1.6.3 Modelling cycle

When developing a model, there are a number of steps that are necessary to undertake, for both IBMs (Grimm and Railsback, 2005) and classical mathematical models (Otto and Day, 2007; Haefner, 2012; EFSA PPR Panel (EFSA Panel on Plant Protection Products and their residues), 2014). Each text gives variations on these steps, and will depend on the type of model being developed, but they broadly involve the following:

1.6.3.1 Formulate the question and hypotheses:

Determine the exact question the model is looking to answer. This could be understanding of a system, or to act as a predictive tool (Caswell, 1976). Having determined the question, use current knowledge, results and theory to determine the possible answers, or hypotheses, to the question. It is these hypotheses that the model is seeking to test.

1.6.3.2 Plan the model:

Using a diagrammatic or qualitative approach lay out the state variables or, in the case of ABMs/IBMs, individuals in the model, and how they will interact with each other and the environment. At this stage it is important to determine the scale at which the model will be implemented, as well as how time will be treated in the model. This will determine the parameterisation required, which will depend on the amount of empirical data available, to at least give realistic ranges for parameters to use. Any lacking data or realistic processes that are too complex to be reasonably included in the model may constrain the design, however this can be a source of inspiration and clear thinking (Starfield, Smith and Bleloch, 1993). This model may be referred to as the *conceptual model* (EFSA PPR Panel (EFSA Panel on Plant Protection Products and their residues), 2014).

1.6.3.3 Implement the model:

In the case of mathematical models, use the qualitative plan of the model to determine the mathematical equations that will make up the model. In the case of IBMs, which will mostly be represented as computer code in a (usually object-oriented)

programming language, determine the schedule of events. As time in a computer simulated model is by necessity discrete, the order in which events occur in each time step is very important. It is then important to design algorithms to capture the behaviour of the system, and to ensure that they are each acting as intended. Once complete, the set of equations or algorithms may be referred to as the *formal model* (EFSA PPR Panel (EFSA Panel on Plant Protection Products and their residues), 2014). It is important to note that this and the previous step will likely be performed many times cyclically, as modelling is an iterative process, design is needed before implementation, but only when the model is implemented is it clear what will and will not work, if the model is behaving as expected and if it is sufficiently, but not over-complex (Grimm and Railsback, 2005). As IBMs do not become more difficult to analyse as complexity increases in the same way as analytical model, it is tempting to include more and more processes, to match the real system as closely as possible. However, unless data is incredibly plentiful and you are able to parameterise the model very precisely, this may not lead to a better model. It is much better to begin with the simplest model possible and increase complexity iteratively. In chapter 3 of this thesis, I present a novel model exploring the in-hive distribution of pesticides and discuss the design and implementation of the model.

1.6.3.4 Analyse the model:

For IBMs or other computer simulated models, the first step is to run the model. This will include parameterising the model using empirical data, and then running sufficient simulations to capture the behaviour of the system. A large number of simulations will be needed either to encompass the range of values each of the

parameters is to take (often a large range due to poor data) or due to randomness in the model, requiring many replicate simulations to ensure that the variability of the model results are captured.

Once the results are obtained, or in the case of analytic models (Haefner, 2012), mathematical analysis has been performed on the model system, it is important to validate the model. Validation of a model can mean a number of different things (Rykiel, 1996). Depending on the type of model, there are a number of approaches and opinions as to whether validation is even possible, and if it is, how best to approach it. Augusiak *et al.* (2014), as part of proposing a strict terminology, refer to the process of ensuring model correctness and quality as ‘evaluation’ (Augusiak *et al.*, 2014), and, choosing to avoid the word ‘validation’ if possible, refer to the process of comparing the model results with real data as ‘model output verification’ when comparing the fit of the model to empirical data and ‘model output corroboration’ when comparing the model output to independent data not used during model development (Augusiak *et al.*, 2014). From here onwards, when using the term ‘validation’, this is what I am referring to, the process of comparing the model’s results to real empirical data, data that was not used to calibrate or parameterise the model. Also important is carrying out a sensitivity analysis of the model (referred to by Augusiak *et al.* as ‘model analysis’ (Augusiak *et al.*, 2014)). This is the procedure of determining how sensitive the model is to alterations of the parameters within the model. This process shows which procedures affect the results most strongly, and may be useful in understanding either the system or model behaviour

These steps will be carried out multiple times as a cycle, so when the model is published and used, more data will be being collected and new insights into the system may be uncovered, which, if the model is to be continued to be used, will need to be considered in further iterations of the model.

In chapters 2 & 4 I present results of a number of simulations using the BEEHAVE model. These results are then analysed and the mechanisms within the model are used to explain the results. In chapter 4, I continue the validation of the BEEHAVE model with respect to varroa, continuing results presented by Becher *et al.* (Becher *et al.*, 2014).

1.7 Honeybee modelling

The honeybee colony is a complex system, existing in a complex landscape, and, as a result the use of models can be of great use to help understand the system and as part of predictive investigation, for example, in risk assessment of pesticides.

A number of models of the honeybee colony have been developed, of many different types (Becher *et al.*, 2013). I will focus on models (or use of models) in three areas: 1) models simulating the honeybee colony to understand the system or as a tool; 2) models looking at the impact of pesticides on the honeybee colony; 3) models looking at the impact of the varroa mite on the honeybee colony, as these are most relevant to my research. Having discussed the historical models, I will then introduce the BEEHAVE model, which is the core of my research.

1.7.1 Existing large colony models

The BEEPOP model (DeGrandi-hoffman et al., 1989) was one of the first and is a computer model consisting of a number of difference equations, which looks at a number of variables such as the weather (wind and rain, for example), the queens reproductive rate to simulate the honeybee colony. It consists of two major sections, one looking at how many eggs the queen lays in a certain period of time and the other tracking those eggs through to adulthood and using the initial weather conditions to calculate foraging activity. It was found from analysis of the model that the biggest impact on the colony dynamics was the potential queen egg laying rate, but there was also quite a serious effect from the availability of spermatozoa to the queen, as, if the queen was not able to fertilize all her eggs, she will lay too many drones and the overall 'workforce' of the colony will be lower. These effects both agree with experiment (DeGrandi-Hoffman *et al.*, 1989)

Schmickl and Crailsheim (Schmickl and Crailsheim, 2007) created a model (HoPoMo) which uses a large number of difference equations to simulate colony dynamics and resource dynamics and also allows the modeller to input a number of environmental parameters to match the landscape a forager would actually meet, giving a large amount of fine control of the environment and processes in the hive and was one of the better tested colony models (Becher, 2013). The large number of variables and processes relating to the 'real environment' are a key feature in more 'lifelike' models like BEEPOP and HoPoMo, that allow more extrapolation to real life.

1.7.2 Modelling of pesticide impacts

Khoury et al. 2011 present a simple dynamical model of the honeybee colony, consisting of two differential equations, modelling the change in worker bee numbers and foragers, with workers being created through an eclosion function and turning into foragers through recruitment and foragers being recruited from the worker population and then dying. The model contains 4 parameters, L – the egg-laying rate of the queen, w – the rate at which the eclosion function approaches the egg-laying rate as the colony size gets large, α – the maximum rate at which workers become foragers, σ – the degree to which the presence of foragers reduces recruitment. Using the model, they predict that an increase in forager deaths can cause younger workers to become foragers, causing stress. This is likely the case (Ushitani *et al.*, 2015). Henry et al. (Henry *et al.*, 2012) use the model, along with empirical work to show that thiamethoxam-induced foraging mortality could cause a colony level impact, however, the parameterisation of the w parameter has been called into question (Cresswell and Thompson, 2012).

Bryden et al. (Bryden *et al.*, 2013) present a mathematical model to assess the degree to which sublethal stress could impact a generic bee colony. This sublethal stress in Bryden et al.'s model is applied as bees becoming impaired and counting less towards calculations of the total population than healthy bees. When analysing the results of the model, Bryden et al. find that as the parameter determining the rate at which healthy bees become impaired, there is a bifurcation in the behaviour of the system, as when it is low, all colonies survive, then it reaches a point at which large colonies

survive and smaller colonies die and then as it increases further, all colonies eventually die. These results imply that even sublethal stresses, if they are widespread in the colony can significantly impair the colony, and cannot be ignored.

Thompson et al. (Thompson *et al.*, 2005) use an existing model(Wilkinson and Smith, 2002), originally intended to study the impact of varroa mites to explore the impact on the colony of a number of empirically determined effects from insect growth regulators (IGRs). They find that application of a period of reduced egg laying is most impactful when occurring in June or August, however, with a sublethal effect of in-hive bees foraging earlier, the most damaging time of application is earlier, in April or May. This highlights the temporal aspect of pesticide risk assessment. It is not only the pesticide that is applied which can affect how a colony will respond, but the timing of the impact that can make a huge difference to the resultant colony health. In chapters 2 and 4 of this thesis, I explore how the timing of a pesticide-induced stress on the colony affects the impact of the stress.

1.7.3 Modelling the impact of varroa

Modelling the impact of the varroa mite on the colony is a more complex task than modelling the impact of pesticides. Both include additional stress to the bees, but in the case of the varroa mite, it is important to also capture the population dynamics of the mite, whose biology is closely linked to the population dynamics of the honeybee, adding complexity. Martin(Martin, 2001) presents a model of varroa and virus

dynamics, with colony dynamics based on BEEPOP (DeGrandi-Hoffman *et al.*, 1989) and a varroa mite model based on earlier work (Martin, 1998) with important changes (including mites still emerging from cells in which the pupae died). In the model there is a variable number of phoretic mites. From this the number of mites invading each brood cell is calculated based on the current population of adult bees, phoretic mites, and brood (Calis, Fries and Ryrie, 1999). When the number of mites within the cells is known, the number of mites produced by reproduction is calculated, and upon the release of the pupae, these mites emerge and become phoretic mites. This model includes two viruses deformed wing virus (DWV) and acute bee paralysis virus (ABPV). He finds that DWV, which has a chronic effect on the bees, reducing the longevity of the individuals which in turn can lead to over-winter colony death as there is no reproduction over the winter period. This is also the impact seen on real colonies that could be attributed, in part, to DWV. ABPV, however, has a much stronger acute effect, killing infected pupae very quickly, leading to colonies dying around September. As ABPV kills the pupae very quickly, it also reduced the mites ability to reproduce. So to have a significant impact on the colony, it requires a large number of infected mites initially.

DeGrandi-Hoffman and Curry (DeGrandi-Hoffman and Curry, 2004) also present a model of varroa dynamics based originally on BEEPOP (DeGrandi-Hoffman *et al.*, 1989), and, as in Martin *et al.* (Martin, 2001), mites infest brood cells based on a functional response table from Calis *et al.* (Calis, Fries and Ryrie, 1999). Whilst in the cell they produce new mites and emerge upon the eclosion of the pupae. When compared to empirical data on the mite drop, a proxy for the mite population within the

colony, the model is close (within one standard deviation of the mean). In 2014 DeGrandi-Hoffman et al. (DeGrandi-Hoffman *et al.*, 2014) use the model to compare to empirical data gathered on bee and mite populations in hives under various miticide treatment scenarios. The model is able to capture the dynamics of the bees very well, however for the mite dynamics, the modelled colonies all had low mite populations at the end of the year, whilst the empirical colonies all gained mites in the autumn. In Chapter 4 of this thesis I will use the same dataset presented in DeGrandi-Hoffman et al. (DeGrandi-Hoffman *et al.*, 2014) and establish the extent to which the BEEHAVE model matches the patterns in the data.

1.8 The BEEHAVE model

Becher et al.(Becher *et al.*, 2013) review a number of models of the honeybee and dynamics of the colony. They find that although there are many models which capture the behaviour they are intending to, there is no model that encompasses many stressors at once. As this is the reality of what the honeybee is subject to in the real environment, this would be an exceptionally useful tool. The BEEHAVE model(Becher *et al.*, 2014) is such a model, it consists of four modules: 1) the colony module – a cohort model with each cohort representing all the bees (separate cohorts for drones and workers) of a certain age, in days. This module calculates the reproduction, mortality and in-hive processes such as brood care and the age at which individual workers become foragers; 2) the foraging module – an IBM modelling the decision-making behaviour and landscape exploitation of the foraging bees, this includes

scouting behaviour and dancing, leading to foragers choosing the most energetically efficient patches in the landscape that the colony is aware of. This module also includes the nectar and pollen flow into the colony; 3) the landscape module – the landscape in the BEEHAVE model consists of a number of patches, defined by their distance to the colony, area and the volume of nectar (including the molar sugar concentration of the nectar) and weight of pollen available on each day. The detection probability (probability that a naïve forager will find the patch) can be set manually or calculated by the model; and 4) the varroa and virus module – based on Martin (Martin, 2001), this module calculates the dynamics of the mites, and also includes the impact of viruses (DWV and ABPV) on the bee population.

As the BEEHAVE model captures a large number of colony dynamics, as well as the landscape and foraging dynamics, it is suitable to answer a large number of questions. Becher et al. (Becher *et al.*, 2014) present simulations using the BEEHAVE model, looking at i) the impact of varroa with an acaricide treatment; ii) how varroa infestation interacts with forage availability; iii) pesticide-induced foraging mortality. Since its publication it has primarily been used to look to simulate effects of modified foraging behaviour and mortality as follows: Thorbek et al. (Thorbek *et al.*, 2016) explore how the BEEHAVE model could be used in establishing the threshold levels used in risk assessment. So far, the European Food Safety Authority (EFSA) have used the model presented in Khoury et al. (Khoury, Myerscough and Barron, 2011), which meets the demands for which it was designed, may be too simple, and misses some vital colony dynamics. Thorbek et al. (Thorbek *et al.*, 2016) find that the landscape quality in terms of forage availability and quality can very much affect the resultant impact of pesticide

induced worker losses. There has also been research by Thorbek et al. (Thorbek, Campbell and Thompson, 2016) using the BEEHAVE model to investigate the extent to which sublethal effects can impact the colony. They simulate two bee-attractive crops, oilseed rape and sunflower and apply sublethal effects of disorientation, reduced food handling ability, and reduced brood care capacity. They find that reduced brood care originating from oilseed rape, with exposure earlier in the year, led to a bottleneck in colony growth and a reduced colony size. In contrast impacts originating from sunflower, with a later exposure did not affect the colony size (as the exposure coincided with peak colony size) but led to a food bottleneck. In all cases, a good landscape can mitigate the impacts. These results imply that if sublethal effects were to be observed to cause colony level impacts, they would be observed in long-term field studies.

Horn et al. (Horn *et al.*, 2016) use the BEEHAVE model to explore how the landscape can impact the colony. Specifically, they are interested in spatial and temporal changes to forage, in the form of distance to forage as well as gaps in forage availability. They find that the distance to the forage has a stronger impact on the number of colonies dying, but gaps in forage led to colony death happening faster.

EFSA performed a review of the BEEHAVE model to assess its suitability as the model to be used in the risk assessment of honeybees to multiple stressors. Although in its current state they find that more work is needed on the model. With the addition of a pesticide module, and confirmation that it captures the impact of the varroa mite

correctly, they suggest that the BEEHAVE model be adopted as the ‘basis for modelling the impact on honeybee colonies of pesticides and other stressors’.

1.9 The main objectives of this thesis

In this thesis, I present research using ecological models to investigate how the honeybee colony responds to a number of stressors, with a focus on simulating simplified impacts of pesticides after forager exposure in the landscape.

The key questions (with the chapters that address them) are:

- A. How does increased mortality of individuals at different life stages (a surrogate for pesticide impact) scale up to colony-level impacts? (Ch. 2 & 4)
- B. How does the individual behaviour of the workers affect likely exposure to pesticides?
 - i) Storage behaviour In the hive (Ch. 3)
 - ii) Foraging behaviour in different landscapes (Ch. 4)
- C. To what extent does the timing of a pesticide exposure affect the resultant impact on the colony?(Ch. 2, 3 & 4)
- D. How well does the BEEHAVE model simulate the population dynamics and impact of the varroa mite, and is it robust to different climates? (Ch. 5)

The specific content of the chapters is as follows:

Chapter 2 - I use the BEEHAVE model to assess how reduced egg-laying rate and increased mortality of the larvae, in-hive workers and foragers affects the colonies. These are surrogates for possible effects of a pesticide and so the mortalities are applied at different times in the year to investigate the effect of the timing of an exposure event. This can help direct risk assessment procedures to the individuals or life stages for which the colony is most sensitive.

Chapter 3 – I present a novel model of the nectar storage behaviour of the colony to assess the extent to which the distribution of pesticide within the colony can impact the exposure of individuals within the colony to pesticides. This model is intended to establish the resolution required when modelling the food storage behaviour and contamination of stored food to attain a conservative exposure estimate.

Chapter 4 – I use the BEEHAVE model to explore the effect of the exposure landscape on the impact of both increased foraging mortality and a sublethal reduction in foraging. To do this, I use two landscapes, one simple and one complex, each with variations.

Chapter 5 – I advance the ‘evaluation’ (Augusiak *et al.*, 2014) of the BEEHAVE model with respect to the impact and dynamics of the varroa mite. Becher *et al.* (Becher *et al.*, 2014) present some initial simulations. I continue these, following

the EFSA evaluation of the model (EFSA Panel on Plant Protection Products and their Residues (PPR), 2015) which suggests that the model underestimates the impact of the mite. To attempt to address this concern, I analyse the impact of three adaptations to the model with the aim of matching the model results to two empirical datasets: one from the UK and one from the USA.

Chapter 2 - Predicting honeybee colony failure: using the BEEHAVE model to simulate colony responses to pesticides

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Predicting Honeybee Colony Failure: Using the BEEHAVE Model to Simulate Colony Responses to Pesticides

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I designed and ran all simulations and analysed the results – with contributing ideas and advice from co-authors.

2.1 Abstract

To simulate effects of pesticides on different honeybee (*Apis mellifera* L.) life stages, the BEEHAVE model was used to explore how increased mortalities of larvae, in-hive workers and foragers, as well as reduced egg-laying rate, could impact colony dynamics over multiple years. Stresses were applied for 30 days, both as multiples of the modelled control mortality and as set percentage daily mortalities to assess the sensitivity of the modelled colony both to small fluctuations in mortality and periods of low to very high daily mortality. These stresses simulate stylised exposure of the

different life stages to nectar and pollen contaminated with pesticide for 30 days. Increasing adult bee mortality had a much greater impact on colony survival than mortality of bee larvae or reduction in egg laying rate. Importantly, the seasonal timing of the imposed mortality affected the magnitude of the impact at colony level. In line with the **LD₅₀**, we propose a new index of 'lethal imposed stress': the **LIS₅₀** which indicates the level of stress on individuals that results in 50% colony mortality. This (or any LIS_x) is a comparative index for exploring the effects of different stressors at colony level in model simulations. While colony failure is not an acceptable protection goal, this index could be used to inform the setting of future regulatory protection goals.

2.2 Introduction

A number of stressors have been implicated in honeybee losses in many parts of the world (vanEngelsdorp and Meixner, 2010) including habitat loss (Naug, 2009); viral diseases (Cox-Foster *et al.*, 2007); parasites such as *Varroa destructor* (Rosenkranz, Aumeier and Ziegelmann, 2010; Annoscia, Del Piccolo and Nazzi, 2012) (which can be a disease vector (Bowen-Walker, Martin and Gunn, 1999; Nordström, 2003; Di Prisco *et al.*, 2011)); and use of pesticides (Johnson, 2015). As all these stressors may interact it is difficult to predict how they change the colony dynamics separately and in combination(s). Moreover, because of the many feedback mechanisms in

honeybee colonies, understanding the relationship between the effects on individuals and the colony level effects is not straightforward. Ecological modelling enables us to disentangle these interactions and explore them both separately, and in combination, in fully controlled simulations. An innate difficulty in studying the effect of pesticides on honeybee colonies is the level of replication needed to capture low level effects at the field scale (Cresswell, 2011). The European Food Safety Authority (EFSA) has described specific protection goals for honeybee colonies stating that “*the magnitude of effects on colonies should not exceed 7% reduction in colony size*” ((Efsa), 2013b). To assess whether this level of impact is occurring a minimum of 60 pairs (control and treatment) of colonies and fields are needed for each study ((Efsa), 2013b). If multiple stressors are to be studied even higher numbers would be needed. Ecological models can help in designing and targeting empirical studies, generating specific hypotheses that later may be tested experimentally, and can be used to assess the risk of environmental chemicals to honeybees ((Efsa), 2013b).

There have been many laboratory, semi-field and field studies showing both acute and chronic effects of pesticides on adult honeybees (Iwasa *et al.*, 2004; Henry *et al.*, 2012; C Sandrock *et al.*, 2014; Cutler *et al.*, 2014; Godfray *et al.*, 2014; Carreck and Ratnieks, 2015; Dively *et al.*, 2015) and bee larvae (Wu *et al.*, 2012; Wilkins *et al.*, 2013). For example, pesticides have the potential to affect foraging via acute mortality (Bailey *et al.*, 2005), or alternatively from sub-lethal effects (Henry *et al.*, 2012; Schneider *et al.*, 2012). Other effects, such as reduced learning acquisition (Tan *et al.*, 2013), decreased rate of learning from olfactory cues (Williamson and Wright, 2013), and reduced communication for recruitment to foraging (Eiri and Nieh, 2012) have

also been shown to occur, but the realism of these exposures is unclear (Carreck and Ratnieks, 2015). In this study, we will concentrate mainly on hypothetical direct lethal effects on different life stages, and reduced egg laying rate that could result from sub-lethal effects on the queen (Dai *et al.*, 2010). This does not capture the complexity of real exposure events, but is important to compare the sensitivity of the colony to mortality of different cohorts at different times of the year in a controlled way.

As pesticides can affect individuals in a number of ways, determining the colony level impact of an individual effect is difficult. Feedback loops may compensate for moderate stresses (e.g. earlier onset of foraging if food stores are low (Huang and Robinson, 1996)) or exacerbate other processes; for instance, less comprehensive care of brood as a result of high in-hive worker mortalities. The BEEHAVE model (Becher *et al.*, 2014) is a suitable tool to investigate this complexity because it integrates in-hive processes and foraging activities to simulate interactions between colony and environment. The model consists of 4 modules (Becher *et al.*, 2014): (i) a landscape module, allowing the user to define a landscape of nectar and pollen in food patches; (ii) a colony module, an age-based cohort model including processes such as nursing and care of brood; (iii) a foraging module, an individual-based model (Grimm and Railsback, 2005) calculating the foraging activities on a particular day and the quantity of both nectar and pollen brought back into the hive; and (iv) a varroa and virus module simulating the population dynamics of the varroa mite and the transmission of viruses. The large number of procedures and feedback loops allow a comprehensive view of the impacts of stressors on the honeybee colony (Becher *et al.*, 2013). Here, we report simulations using the BEEHAVE model (Becher *et al.*,

2014) to explore the colony-level impact of altering the mortality of a number of honeybee life-stages and reducing the egg-laying rate of the queen at different times of the year.

Since such simulations enable the user to examine a whole variety of stressors on individual bees, and the effects on the colony, finding a standard way of comparing the responses of the colony would be useful in risk assessment. For environmental chemicals the **LD₅₀** is the standard index used to describe the median lethal dose of a toxin i.e. that resulting in 50% subject mortality (Trevan, 1927). Here we present an index to compare the impact of different imposed stresses on colony survival, the **LIS₅₀**, describing the ‘Lethal Imposed Stress’ level resulting in a 50% colony mortality as predicted using the BEEHAVE model. We also present the **LIS₁₀** which predicts 10% colony failure from an imposed stress. We argue that these indices will be useful for comparing the impact of imposed stressors at colony level; and could also inform the setting of pesticide protection goals in future, once the indices have been applied to a wider variety of stressors and their variability has been quantified.

2.3 Methods & Model

2.3.1 Model Parameterisation

The BEEHAVE model (Becher *et al.*, 2014) (BEEHAVE-Model Version 2014-03-04, free to download at www.beehave-model.net) was modified to increase the daily mortality of different life stages of bees in the colony from a defined day for a

defined period to simulate potential effects of an exposure event (where exposure is defined as the period when toxic effects are imposed). We used the "default" setting as described in Becher et al. (Becher *et al.*, 2014), altering the landscape as explained below.

The simulations started from 1st January with 10,000 worker bees in a colony and ran for three years with each year having an identical annual weather cycle (based on maximal temperature and hours of sunlight at Rothamsted Research, Hertfordshire, UK in 2009). The colony was free from varroa and disease, as the purpose of the simulations was to look at effects of singular events increasing mortality in isolation. On the last day of each year, if there were fewer than 4000 adult bees present, the colony is assumed to die due to winter mortality (Becher *et al.*, 2014). At the end of each three year simulation the number of bees alive in the colony, or alternatively whether the colony had failed, was recorded.

2.3.2 Landscape

BEEHAVE allows users to define a dynamic landscape, giving values for the distance of each food patch to the colony, and the nectar quantity (L), nectar quality (sucrose concentration (mol/l)) and pollen quantity (kg) for each food patch on each day of the year. The simulations were set up in a very simplified and stylised modelled landscape: there was a single food patch 1km away from the hive offering 20L of nectar and 1kg of pollen each day of the year (although not representing the

complexity of real landscapes, this enables tests of potential “exposure” in each month of the year in a controlled manner).

2.3.3 Imposed stress

We ran simulations to contrast the effects of five different imposed stresses: reduced egg laying rate (ELR) of the queen, increased daily larval mortality, increased daily in-hive worker mortality, and increased forager mortality, applied daily or applied on each foraging trip. In reality an exposure event may affect a combination of life stages over varying timeframes via different routes (nectar, pollen, honey, wax) but to specifically examine the sensitivity of different life stages we chose a simplified set of simulations: examining increased mortality of individual life stages, during single exposure periods, when that exposure is assumed to be direct via consumption of nectar and pollen. We also ran the simulations for one combination of daily life stage mortalities (larvae, in-hive workers, foragers). Pupal mortality was not tested as the pupae are in capped cells and not receiving food and therefore are unlikely to be exposed directly via nectar and pollen). In each simulation a single stressor was applied for a continuous 30 day period each year. Duration of bloom of different crops differ widely as do persistence of different pesticides, but we here chose a 30 day exposure period as typical. Timing-dependent effects were investigated by running scenarios with the 30 day exposure period beginning on the first day of each month of the year. Imposed mortalities were applied as both multiples of the control value in the model and as set percent daily mortalities, while reduced egg-laying rate was only applied as a percent reduction.

Testing a multiple of the control reflects the typical procedure of pesticide risk assessments¹¹. We also ran simulations with 'set percent daily mortalities' to determine the actual percentage of increased mortality that the colony could withstand.

2.3.3.1 Egg-Laying Rate

The daily egg-laying rate varies seasonally depending on the day of the year (for distribution see Becher et al. (Becher *et al.*, 2014)), and eggs are lost at a rate of ~3% per day by default (Becher *et al.*, 2014). For the simulations, the egg laying rate (number of eggs produced on a particular day) was reduced by 25%, 50%, 75% and 90% for a period of 30 days with zero reduction applied as a control.

2.3.3.2 Stage-Specific Daily Mortalities

The daily mortalities of the larvae, in-hive workers and foragers were altered in two ways:

1. Control daily background mortality was multiplied by a factor of 1, 1.5, 2, or 3.
2. Daily background mortality was set to a set percentage during the treatment period: one of 1%, 5%, 10%, 25% and 50% each day.

The control daily background mortality is typically low: ~0.4% for the in-hive workers²⁸ and foragers and ~1% for the larvae²⁸. For the larvae this mortality does not include the chance of dying from lack of food or brood care.

2.3.3.3 Forager Mortality During A Foraging Trip

Increased foraging trip mortality was simulated in two ways, similar to the stage-specific daily mortality simulations: (i) control value was multiplied by 1 (control), 1.5, 2 or 3; and (ii) set values of 1%, 2.5%, 5%, 7.5% and 10% mortality per trip. Lower values than for the daily mortalities were used because foragers take multiple trips on a single day, so the majority of foragers may die if a forager had a 25% or 50% chance of dying on each trip, reducing the impact of higher mortalities as each bee can only die once. These settings were used to simulate pesticide exposure at levels high enough to cause death in the foragers through either immediate acute mortality, gradual weakening during the return flight, or through behavioural changes leading to impaired orientation and consequent homing failure (Henry *et al.*, 2012). Foraging mortality in the model depends on the duration of a foraging trip and is applied before an individual forager returns to the colony. For the single food patch present in the simulations the mortality is ~1.5% for nectar foragers and ~0.9% for pollen foragers under control conditions (values taken from BEEHAVE model during control simulations), Although these values can vary during a day (as handling time of a food patch is increased when the patch is depleted), enough nectar and pollen are provided at the patch that this variation is negligible.

2.3.3.4 Simultaneous daily mortality of larvae, in-hive workers and foragers

To simulate an event in which several life stage are affected, the mortality of each of the larvae, in-hive workers and foragers were all modified simultaneously in two ways:

1. Control daily background mortalities of larvae, in-hive bees and foragers were multiplied by a factor of 1, 1.5, 2 or 3.
2. Daily background mortalities of larvae, in-hive bees and foragers were set to a set percentage during the treatment period: one of 1%, 5%, 10%, 25% and 50% each day.

While it is unrealistic that different life stages are affected with identical effect levels, this scenario demonstrated the colony's sensitivity to multiple effect types.

Each scenario was run for 30 replicates, with the mean number of live bees at the end of three years as output. For each combination of mortality type and 30 day exposure period, a linear regression was carried out between either the factor increase of the control or the percentage imposed mortality per bee and the mean number of bees alive per colony at the end of the 3 year simulation. The slopes of these regressions were plotted (Figure 2-1), showing how sensitive the colony is to increased mortality of each life stage. The actual changes in bee numbers from which the slopes are calculated are plotted in Figs 2-2, 2-3,& 2-4.

2.3.4 LIS₅₀: An index for comparing lethal imposed stresses at colony level

To compare between the effects of these imposed stresses at colony level, we calculated a new index analogous to the LD₅₀: the LIS₅₀ (Lethal Imposed Stress) was calculated as the level of imposed stress at the individual level that led, statistically, to 50% of the colonies dying in the BEEHAVE simulations within three years (using a threshold for survival of at least 4000 bees alive on the last day of each year) (Becher *et al.*, 2014). In these simulations, the level of imposed stress was the percentage stage-specific daily mortality or percentage chance of dying during a foraging trip. The LIS₅₀ was calculated using the “dose.p” function in R’s MASS library on a generalised linear model (GLM) built using data on the number of colonies alive after increased mortalities were imposed. For each of the imposed stresses in question, the mortality was applied from 0% to 100% (in 5% increments) for each month of the year (separately) with 50 replicates. For foraging mortality per trip, preliminary runs showed that colony death occurred when foraging mortality was 40% for all tested months, so higher mortalities were not tested.

The LIS₅₀ was chosen for its theoretical parallel to the LD₅₀, but a LIS_x could be calculated for any percentage of colony failure (x) that is of interest e.g. LIS₁₀ figures are also presented, predicting the level of stress resulting in 10% colony deaths.

2.4 Results

2.4.1 Reducing egg-laying rate (ELR)

Reducing the ELR for 30 days had only a moderate impact with none of the colonies dying in any of the simulations (Figure 2-2). A reduction of the daily egg laying rate by 90% (i.e. to 10% of the control) in June led to the average colony size at the end of three years being reduced by 35% of the initial population (Figure 2-2). Between April and August, each percent reduction in ELR led to only 50 fewer bees per colony after three years (Figure 1A). Nevertheless, colony dynamics was affected to varying degrees depending on the season and the reduction in the egg laying rate (Figure 2-2).

2.4.2 Effect of increasing mortality as multiple of the control

The colony was not highly sensitive to an increase in daily mortality of the larvae or the in-hive workers within the tested range (Figure 2-1B; Figure 2-3 A + B). For the larvae, the control background mortality was already low (~1%) and the majority of larvae that died in the control simulations did so from a lack of resources (food or brood care). Therefore, small larval losses from increased background mortality could be compensated in the model by allowing resources to be spread amongst remaining larvae, reducing mortality from a lack of these resources. As with background larval mortality, the control value of daily in-hive worker mortality in the model is small (~0.4%), such that trebling it equates to 1.2% daily mortality and does not result in large losses over the course of the month. The impact of increasing the control daily

forager mortality was low when imposed in January to August, but the colony was sensitive to increased mortality imposed in September to December (Figure 2-1B; Figure 2-3 C). The critical threshold for colony survival in the BEEHAVE model was applied on the last day of December; therefore the colony had the whole year to recover from increased individual mortality applied in January, before winter survival was calculated. When the increased foraging mortality was applied to foragers at the food patch on each successful foraging trip, the impact on the colony was much larger (colony reduced down to almost 4,000 bees in June) (Figure 2-1C; Figure 2-3 D), as the mortality was applied many times per day and background mortality is higher than for in-hive life stages. This impact on the colony was likely due to the decreased food stores in the colony. These effects were particularly strong if the stress was imposed during the summer months when foragers were making the most foraging trips. For simulations multiplying the stage specific control mortalities, per-trip foraging mortality was the only single imposed stress to lead to colony failure with 3x mortality leading to 77% colony survival (Table 1-3). When the mortality of larvae, in-hive workers and foragers were applied simultaneously as a multiple of the control mortalities, the impact on the colony was similar to the worst case equivalent single mortality (Figure 2-1B, Figure 2-5), the single life-stage daily mortality to which the colony is most sensitive when applied as a multiple of default.

2.4.3 Effect of increasing mortality by set percentage

The largest impact on the colony from larval mortality came when the effect was applied in one of the months between April and August (Figure 2-1D&E; Figure 2-4A). During this period, the colony has a lot of larvae as it is building to peak numbers and increased mortality reduced or delayed this peak (Figure 2-6). Very high larval mortality in May and June led to the colony population being reduced to between 2000-3000 individuals and winter mortality was high (Figure 2-4A). When larval mortality was increased (illustrated in Figure 2-6 for a level of 25% daily mortality), the resulting loss of larval numbers had the effect of reducing deaths due to lack of food or care during the treatment period, as these became more readily available for the surviving larvae. This feedback allowed the colony to compensate for moderate increases in larval mortality. However, high larval mortalities during summer led to a reduction in the worker population, which in turn led to a further peak in larval mortality one week after the end of the treatment period (Figure 2-6).

The modelled colony was sensitive to losses of adult bees in most months (Figure 1D&E; Figure 2-4 B-D Figure 2-7, Figure 208). With respect to daily mortalities, the colony was more sensitive to losses of the younger in-hive workers than to the older foragers. During the period of April to September, the same period in which brood mortalities had a noticeable impact (Figure 2-1D; Figure 2-4), a daily in-hive worker mortality of over 25% led to all colonies being lost between May and July (Table 2-1) and a 5% daily mortality led to up to 40,000 more in-hive worker deaths over the course of the month (Figure 2-8). Loss of in-hive bees led to a large increase in brood loss from lack of care or food over the rest of the year (Figure 2-7C), while also reducing

the honey stores in the hive (Figure 2-7E). This combined stress was very damaging to the colony. Outside spring and summer, high daily forager mortality was devastating to the colony (Figure 2-1D; Figure 2-4C). This is because during the autumn and winter only few eggs are laid, so, the colony consists primarily of older bees still termed “foragers” (even though they rarely exited the colony).

The results of these simulations also highlighted the potential sensitivity of the colony to patch-specific forager mortalities, experienced on each foraging trip (Figure 2-1E). There was little effect at the very beginning or end of the year due to the lack of foraging activity at these times. Between May to October, there was a large impact on the colony from increasing this foraging mortality (with June being the most sensitive month, as there was more time to forage in June than other months). A 5% mortality at the food patch applied in June led to an average colony size of ~1000 bees at the end of the year (Figure 2-4D) with only 5 of 30 replicate colonies surviving (17% Table 2-1).

When the mortalities were applied as a set percent to several life stages simultaneously, this had a consistently higher impact than the worst case individual daily life stage mortality in each particular month (forager mortality in winter and in-hive mortality in summer) (Figure 2-1D). When colonies were subjected to combined daily mortalities of over 10% at any time of the year, then no colonies survived (Table 2-1).

	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec
Set Percent												
Daily Larval Mortality												
1%	-	-	-	-	-	-	-	-	-	-	-	-
5%	-	-	-	-	-	-	-	-	-	-	-	-
10%	-	-	-	-	-	-	-	-	-	-	-	-
25%	-	-	-	-	-	-	-	-	-	-	-	-
50%	-	-	-	90%	20%	50%	80%	-	-	-	-	-
Daily In-Hive Mortality												
1%	-	-	-	-	-	-	-	-	-	-	-	-
5%	-	-	-	-	-	-	-	-	-	-	-	-
10%	-	-	-	-	-	13%	97%	-	-	-	-	-
25%	-	-	-	-	0%	0%	0%	10%	33%	-	-	-
50%	-	-	-	-	0%	0%	0%	3%	47%	-	-	-
Daily Forager Mortality												
1%	-	-	-	-	-	-	-	-	-	-	-	-
5%	-	-	-	-	-	-	-	-	-	0%	0%	0%
10%	20%	27%	77%	-	-	-	-	-	37%	0%	0%	0%
25%	0%	0%	0%	97%	---	7%	53%	93%	0%	0%	0%	0%
50%	0%	0%	0%	97%	67%	0%	0%	7%	0%	0%	0%	0%
Per-Trip Forager Mortality												
1%	-	-	-	-	-	-	-	-	-	-	-	-
2.5%	-	-	-	-	-	-	-	-	-	-	-	-
5%	-	-	-	-	-	17%	87%	-	-	-	-	-
7.5%	-	-	-	-	97%	0%	10%	77%	97%	-	-	-
10%	-	-	-	-	77%	0%	0%	27%	83%	40%	-	-
Combined Mortality												
1%	-	-	-	-	-	-	-	-	-	-	-	-
5%	-	-	-	-	97%	97%	97%	80%	0%	0%	0%	0%
10%	0%	0%	0%	27%	0%	0%	3%	0%	0%	0%	0%	0%
25%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
50%	0%	0%	0%	0	0%	0%	0%	0%	0%	0%	0%	0%

Table 2-1

Percentage of the 30 replicate colonies surviving 3 years of an imposed stress of a set percent mortality on one life stage for one month of the year (- represents 100% survival). A colony that has more than 4000 bees at the end of three years is assumed to survive the winter.

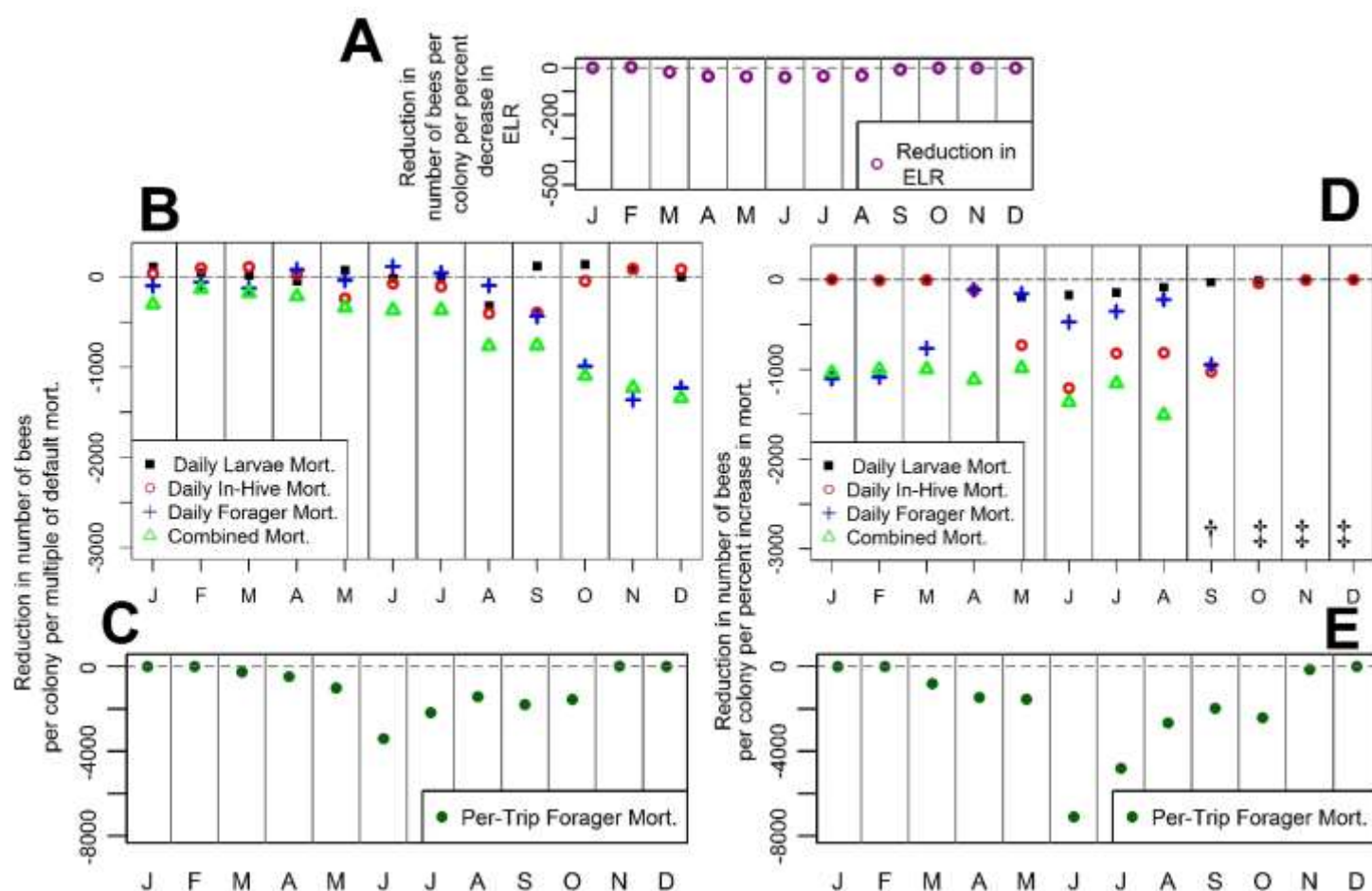


Figure 2-1

Calculated sensitivity of colonies to each stage mortality imposed for 30 days, calculated as the slope of the linear regression for the simulation data shown in Figures 2-2, 2-3, & 2-4. For each combination of imposed stress and treatment month, a linear regression was performed with the colony population at the end of the third year against the magnitude of the imposed stress. The graphs show the reduction in colony size A) per percent decrease in egg-laying rate (ELR); B) per multiple of the control background daily mortality for larvae, in-hive bees and foragers; C) per multiple of

control background per-trip mortality; D) per percent daily mortality of larvae, in-hive bees and foragers; and E) per percent daily per-trip mortality.

† In these months, all levels of the combined mortality except 1% mortality lead to all colonies being lost, therefore it was not possible to fit a linear regression.

‡ In these months, all levels of both the forager and the combined mortalities except 1% mortality lead to all colonies being lost, therefore it was not possible to fit a linear regression

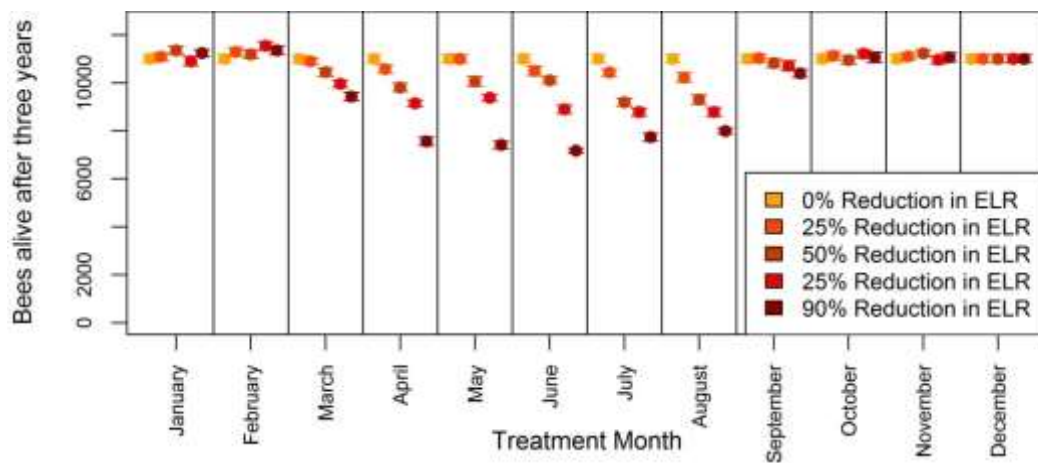


Figure 2-2 Reducing egg-laying rate

The mean number of bees alive in a colony (\pm standard error) at the end of three year simulations ($n = 30$) when the colony is subject to a reduction in the egg-Laying rate(ELR) for one month in the year.

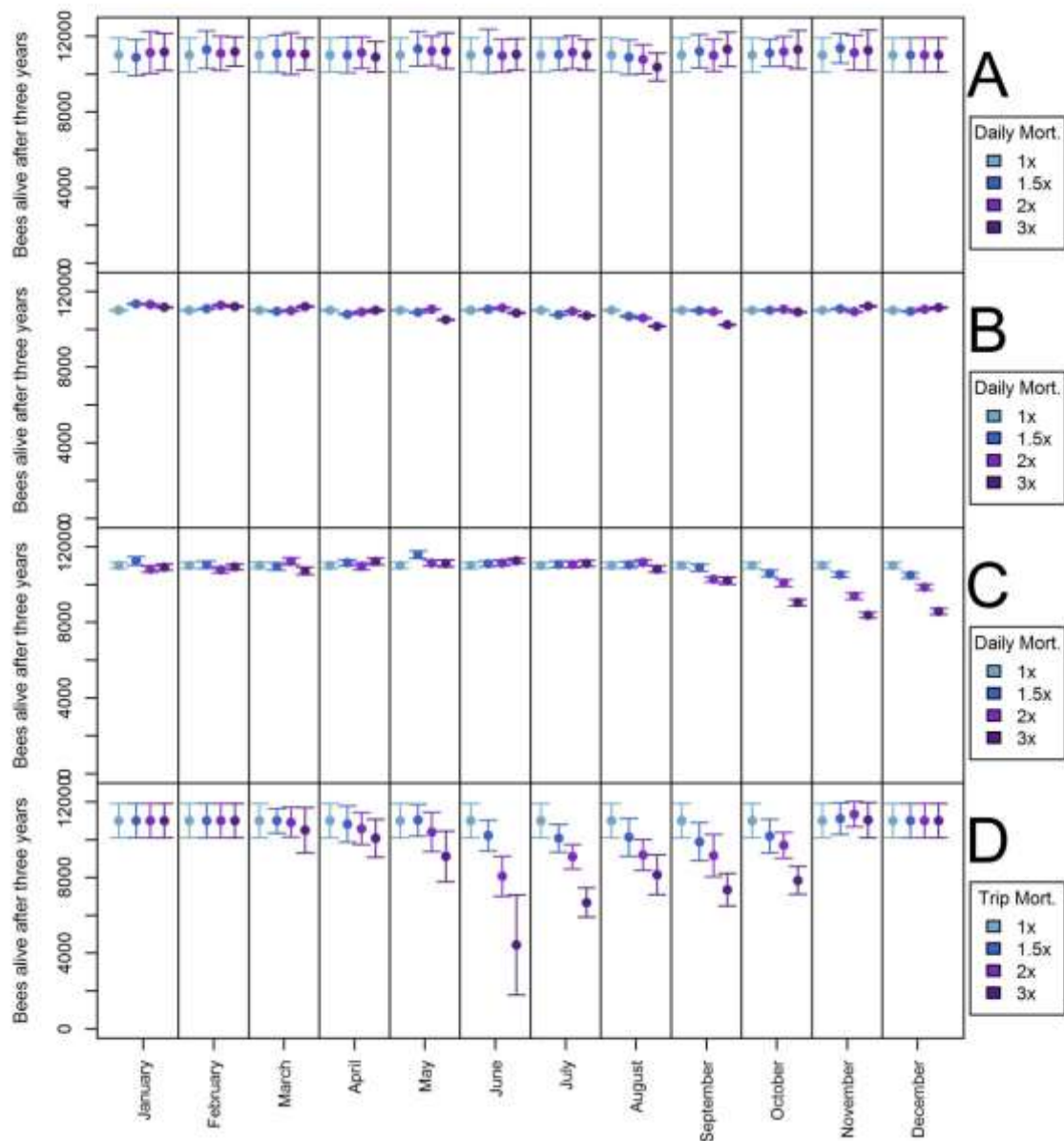


Figure 2-3 Increasing mortality as a multiple of control

The number of bees alive in the colony (\pm standard error) at the end of three year simulations ($n = 30$) when the colony is subject increased mortality, (multiple of the model control mortality) of certain life stages for one month in the year. The standard error of the mean is shown.

- A: Larval daily mortality
- B: In-Hive worker mortality
- C: Forager daily mortality
- D: Forager per trip mortality.

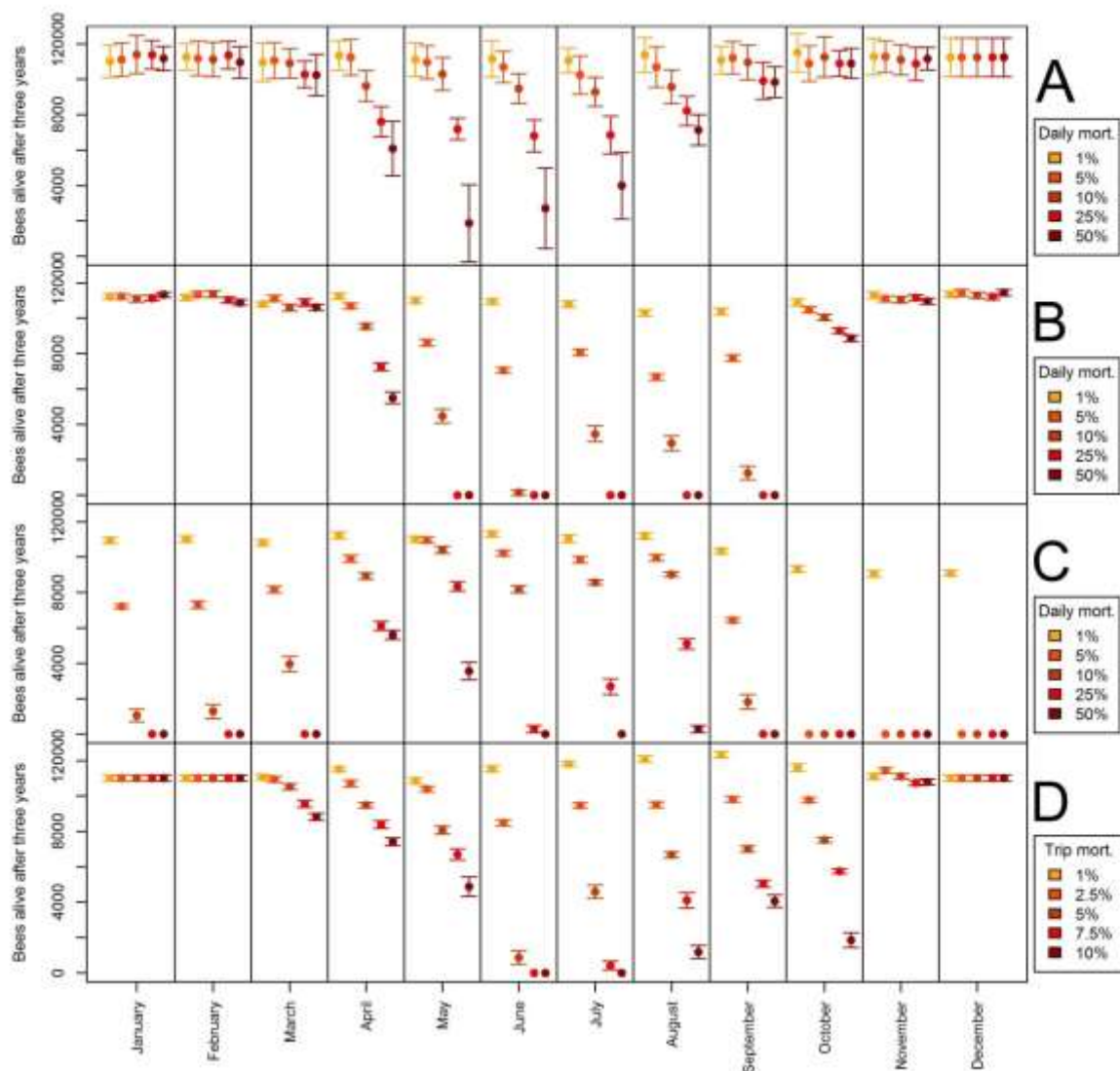


Figure 2-4 Increasing mortality by a set percentage

The number of bees alive in the colony (\pm standard error) at the end of three year simulations ($n = 30$) when the colony is subject increased mortality (set percentage mortality) , of certain life stages for one month in the year. The standard error of the mean is shown.

A: Larval daily mortality

B: In-Hive worker daily mortality

C: Forager daily mortality

D: Forager per trip mortality

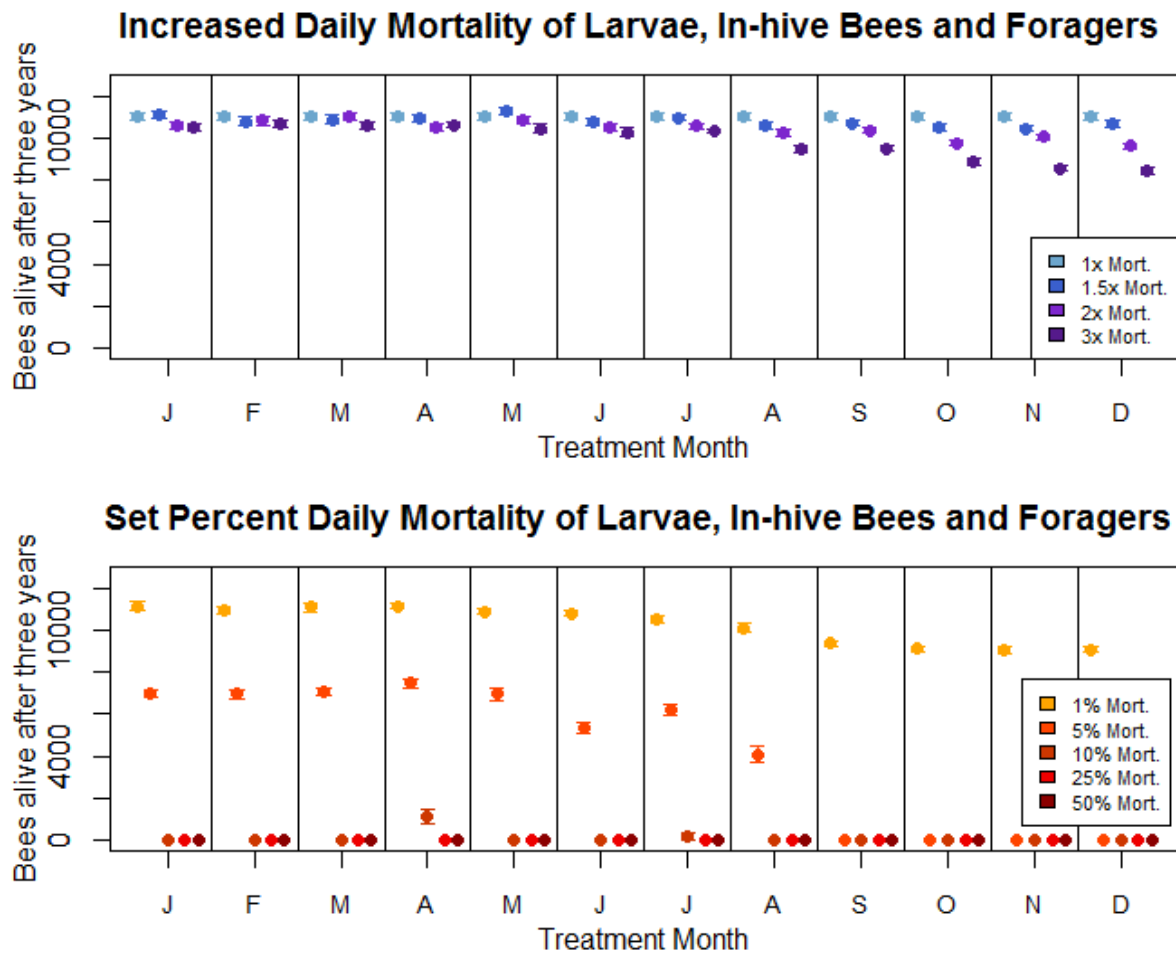


Figure 2-5

The number of bees alive in the colony (\pm standard error) at the end of three year simulations ($n = 30$) when the colony is subject increased mortality (set percentage mortality) , of larvae, in-hive workers and foragers for one month in the year. The standard error of the mean is shown.

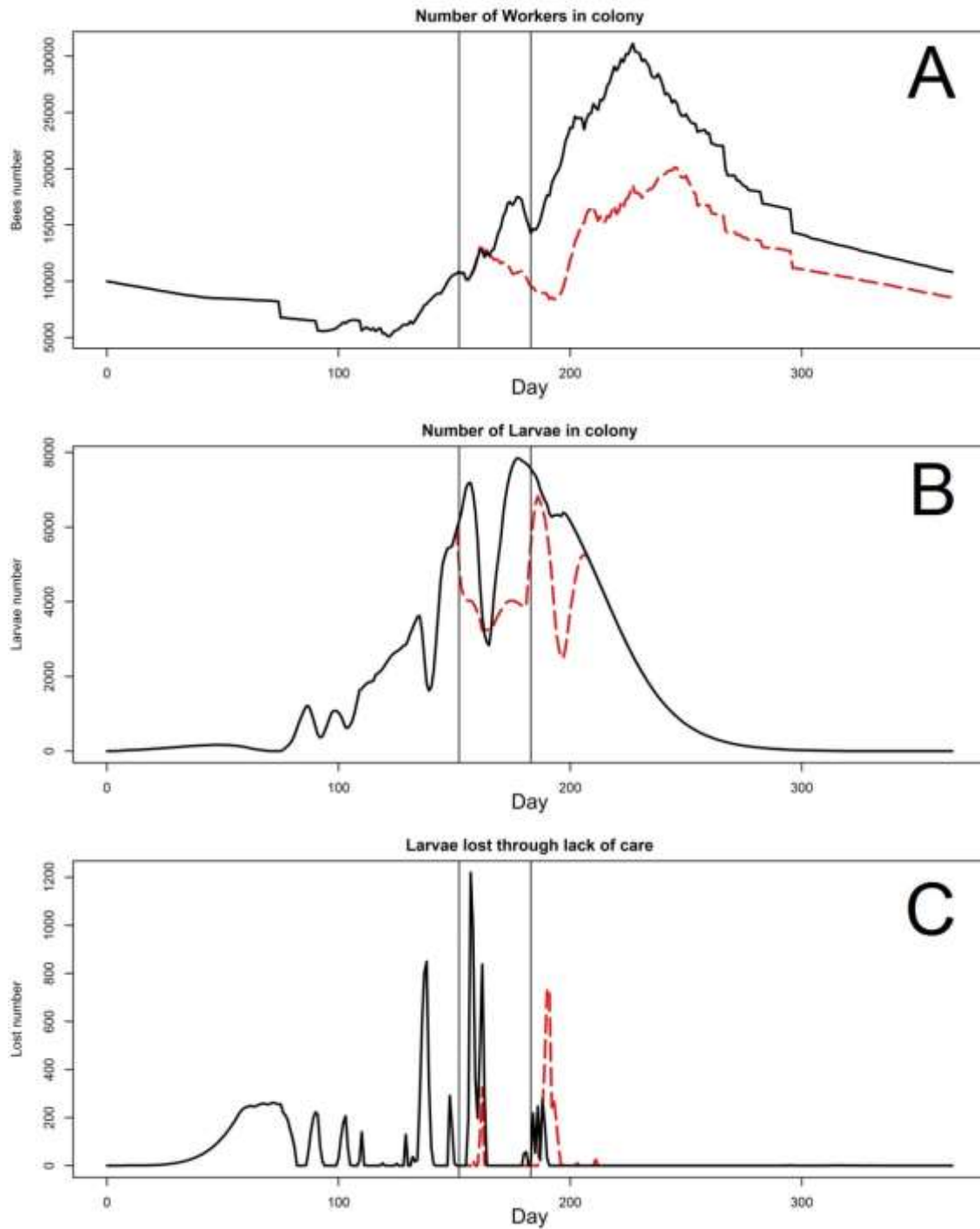


Figure 2-6

Detailed effects of imposing 25% daily larval mortality in June. Black solid line represents the control scenario and the red dashed line represents the treatment scenario. The treatment period is between the vertical lines. Data was collected from BEEHAVE simulations set up exactly as described in the methods, taking values at the end of each day in the model.

A: Number of Workers in Colony

B: Number of Larvae in colony

C: Number of larval deaths from lack of food or brood care

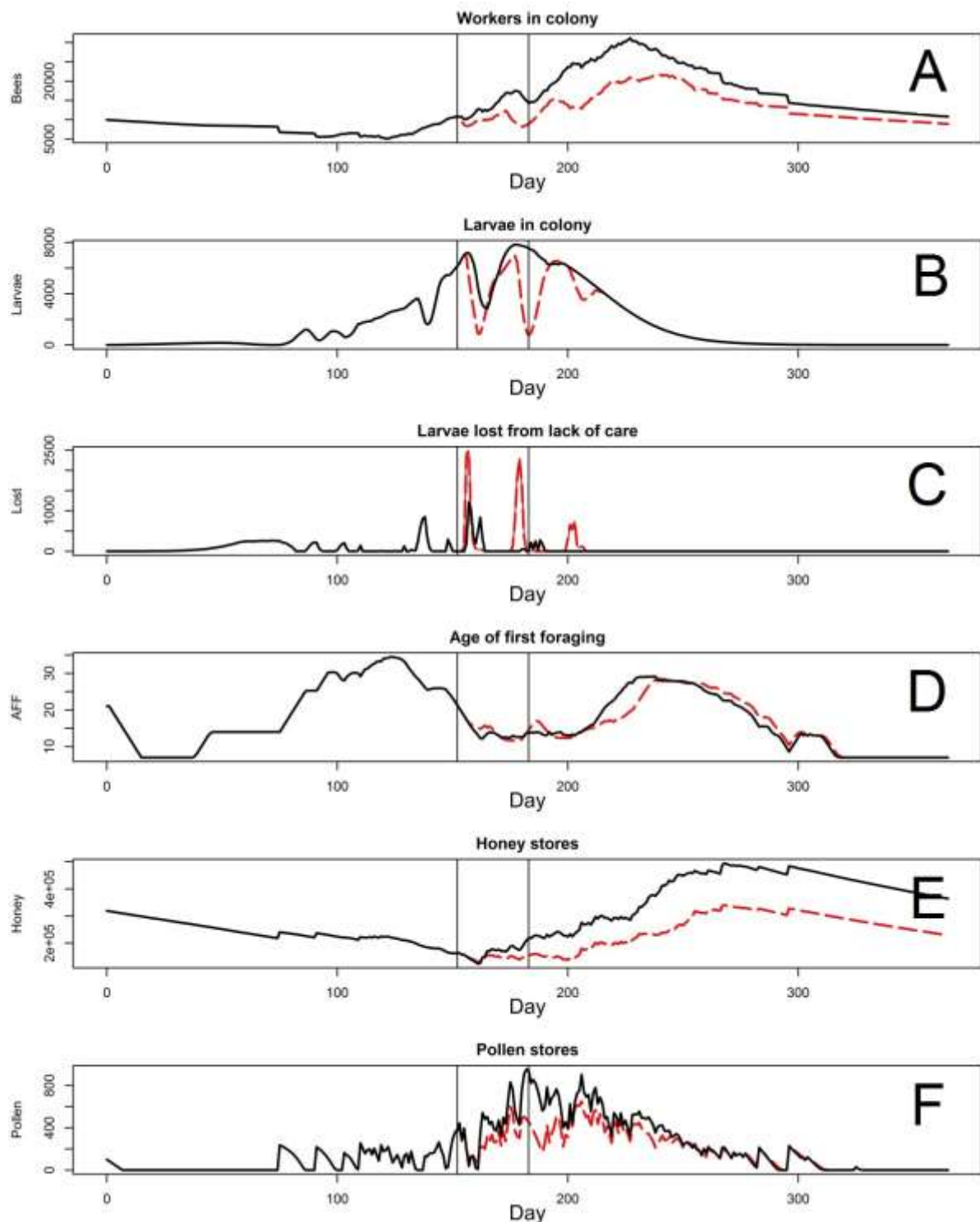


Figure 2-7

Detailed effects of imposing 5% daily in-hive worker mortality in June. Black solid line represents the control scenario and the red dashed line represents the treatment scenario. The treatment period is between the vertical lines. Data was collected from BEEHAVE simulations set up exactly as described in the methods, taking values at the end of each day in the model.

A: Number of workers in colony; B: Number of larvae in colony; C: Number of larval deaths from lack of food or brood care; D: Age when workers first become foragers (days); E: Honey store of the colony (J); F: Pollen stores in the colony (g)

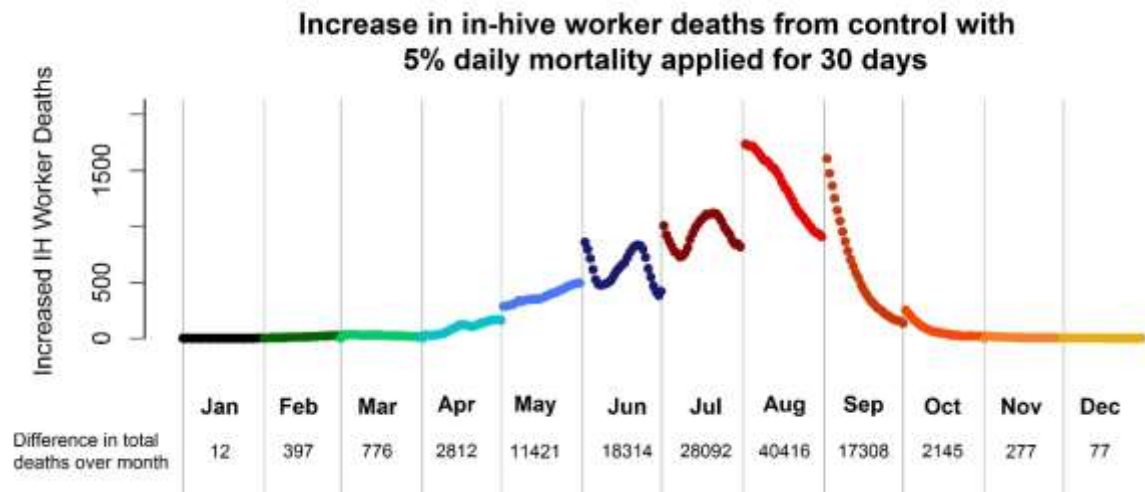


Figure 2-8

The increase in in-hive worker deaths in the colony after the addition of an extra 5% daily in-hive worker mortality in a month compared to the control. The points show the increase in number of in-hive worker deaths each day during the treatment month compared to the same month in a control simulation. The values below each month give the total difference in in-hive worker deaths for the month in question.

2.4.4 LIS₅₀: An index for comparing the lethality of different stressors on colonies

The LIS₅₀ values represent the statistical likelihood that a certain imposed stress will lead to 50% colony failure for the specific control conditions used in the model (in this case calculated after 3 years). With agreement on appropriate control settings, the LIS₅₀ could be standardised for use over any number of months or years, depending on the sensitivity required.

Table 2-2 contains LIS₅₀ values for four stressors imposed for 30-day exposures in four different months. The months were chosen to be those when foragers are active, crops flower and the colony is therefore most likely to be exposed. A low value indicates that a low daily percentage mortality imposed on individuals led to high colony failure and, therefore, identifies stressors to which the colonies are most sensitive. No values for the reduction in egg-laying rate were given as no 30-day reduction of egg-laying rate led to colonies dying for any of the chosen months.

Table 2-2

Daily percentage mortality of specific honeybee life stages required over 30 days to statistically kill 50% and 10% (LIS₅₀ and LIS₁₀) of colonies over three years in an otherwise beneficial environment (ample food and no pathogens) \pm standard error. Values of >100% imply that in all of the simulations, 50% colony loss was not reached. No simulations imposing reduced egg-laying rate lead to colony loss.

	Larvae mortality per day		In-hive mortality per day		Forager loss per day		Forager loss per trip	
	LIS ₅₀	LIS ₁₀	LIS ₅₀	LIS ₁₀	LIS ₅₀	LIS ₁₀	LIS ₅₀	LIS ₁₀
April	>100%	76% \pm 3%	>100%	76% \pm 5%	>100%	68% \pm 3%	23% \pm 0.4%	18% \pm 0.5%
May	47% \pm 1%	33% \pm 1%	11% \pm 0.2%	9% \pm 0.3%	68% \pm 1%	49% \pm 2%	16% \pm 0.4%	8% \pm 0.7%
June	63% \pm 1%	31% \pm 2%	7% \pm 0.1%	6% \pm 0.2%	20% \pm 0%	16% \pm 0.7%	4% \pm 0.1%	3.6% \pm 0.1%
July	81% \pm 1%	38% \pm 5%	11% \pm 0.2	9% \pm 0.2%	25% \pm 1%	18% \pm 0.9%	6% \pm 0.2%	5% \pm 0.3%

The colonies were most sensitive to in-hive worker daily mortality and to per-trip foraging mortality (Table 2-2, Figure 2-1, 2-9). The in-hive worker mortality started to become very influential in the period between April and May, when the respective LIS₅₀ went from over 100% in April to 11%, in May and 7% in June (Table 2-2, Figure 2-9) as the brood nest was growing exponentially, requiring a large workforce of nursing bees, and the colony structure moved from mostly foragers (the over-winter bees) to more younger in-hive workers. The LIS₅₀ for the forager mortality per trip has a relatively low value during the summer, with a value of just 4% in June. Such mortality was applied many times a day to foragers, especially those that were particularly active within a treated patch so the cumulative daily mortality was higher. The colonies were also sensitive, but to a lesser extent, to daily forager and larval mortality. Daily forager mortality had the largest impact in June and July, larval mortality in May and June.

Importantly, the different imposed stresses had their greatest impact (smallest LIS₅₀) at different times of the year. Specifically, larval mortality had the lowest LIS₅₀ in May, whereas the two forager mortalities and the in-hive mortalities were at their lowest in June. The reason was that increased larval mortality led to a reduction of the adult in-hive population later in the year (Figure 2-6A) therefore reducing larval population in May could lead to reduced in-hive worker population in June (and all impacts which arise from that).

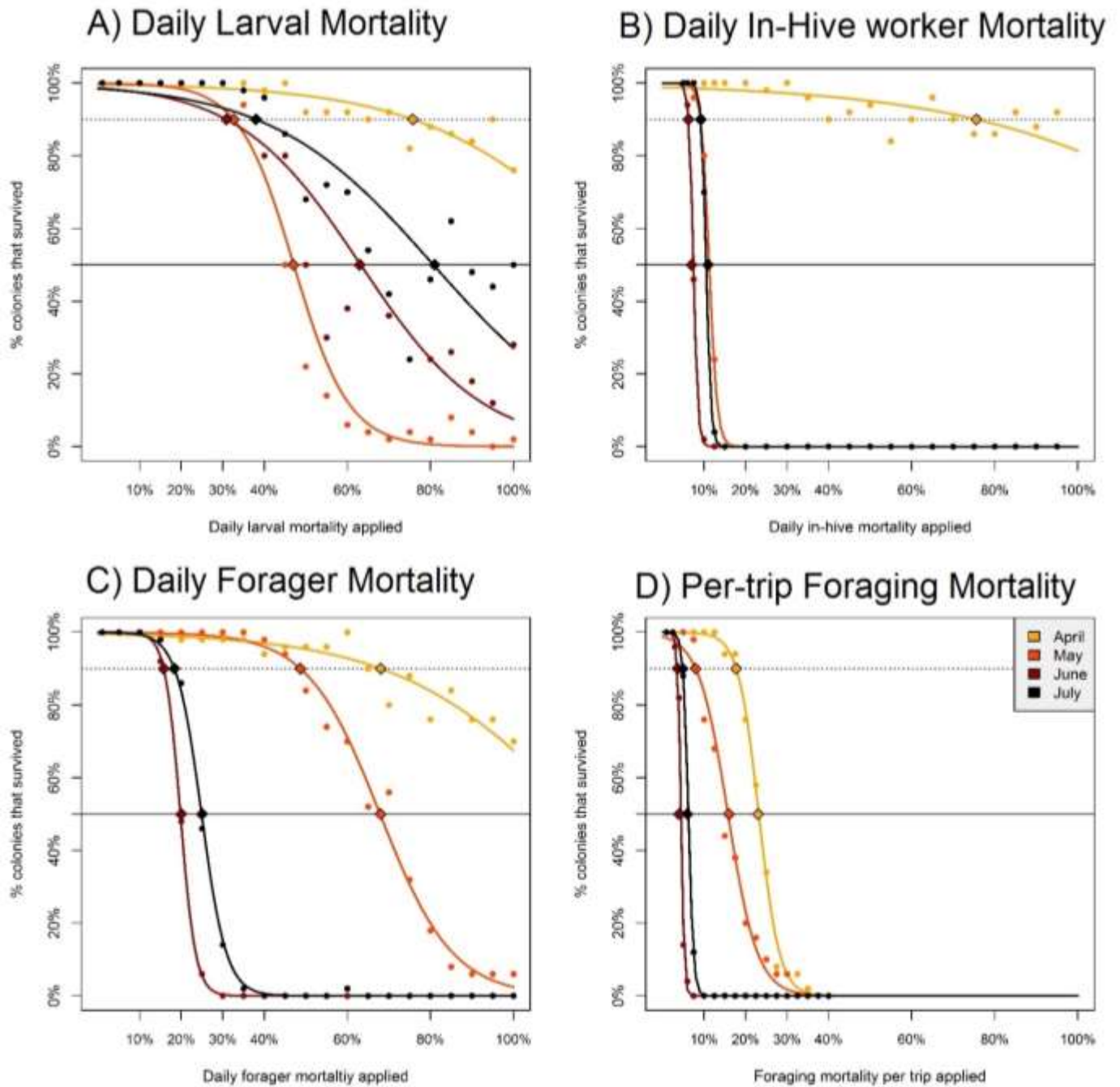


Figure 2-9

Imposed-stress-response curves for the four mortalities investigated: (A) daily larval mortality, (B) daily in-hive worker mortality, (C) daily forager mortality, and (D) forager mortality per foraging trip. These show the % survival of 30 colonies for each of the

varying mortalities, at the end of three years. Different coloured lines are shown for these mortalities applied for 30 days in April (yellow line), May (orange line), June (red line) or July (black line). The intercept between a response curve and the solid horizontal line indicates its LIS_{50} , [Table 2-2], while the intercept with the dashed horizontal indicates its LIS_{10} .

Alternative thresholds of colony failure may be explored with LIS_x : so LIS_{10} values have also been included in Table 1 showing the level of stress causing 10% colony failure. Although the LIS_{10} is likely to be quite variable, it gives useful information when used in conjunction with LIS_{50} : for certain life stages in certain months (in-hive bees and foraging trip mortality), LIS_{10} and LIS_{50} were remarkably similar (Fig 2-9B; 2-9D) where the stress response curve was so steep that there were effectively tipping points when any increase in daily mortality rates (imposed for this period of 30 days) led to all colonies failing.

2.5 Discussion

Using a set of simplified scenarios, our results showed a large variation in the impacts of imposed stress on the honeybee colony depending on both the demographic stage targeted by the imposed stress and the time of year in which the stress is applied. Imposed stress on all stages, except for daily forager mortality, led to highest colony losses from April to August. Imposed stress to the adult workers was, most often, more damaging to the colony than effects on the brood; and imposed stress applied to in-

hive workers had a larger impact in late summer than the rest of the year. This was partly a consequence of the large proportion of in-hive workers, and that individuals were in-hive bees for a relatively long time such that cumulative stage mortality was higher, even if daily mortality was the same as for other life stages. In-hive bees (Table 2) would have consumed a lot of resources in their development but as yet not started generating them for the colony by foraging for nectar and pollen.

Table 2-3

Characteristics of specific life stages used in the BEEHAVE model relating to life stage duration, food requirement and major source of mortality. Values are parameters set in the model (Becher et al. 2014)

** In-hive bees will become foragers after between 7 and 50 days depending on colony conditions*

† Nectar consumption increases with level of brood care

Life-stage	Time spent in stage	Honey Requirement (mg/day)	Pollen Requirement (mg/day)	Major source of mortality
Egg	3 days	0	0	Lack of care
Larva	6 days	10.9	23.6	Lack of food/care
Pupa	12 days	0	0	Lack of care
In-Hive Worker	Variable* (mean 17 days)	11(53.42)†	1.5	Background mortality
Forager	Until death (Summer average 24.5 days Winter average 143 days)	11 + for foraging	1.5	Foraging mortality

When the stress was imposed as a multiple of the default, there was little impact on the colony, except in the case of mortality calculated per foraging trip. This suggests that similar fluctuations in daily mortality are insufficient to cause colony loss in isolation, whereas foraging mortality calculated on a per trip basis carries a higher risk to the colony because a forager can perform many trips per day. When mortality of a life stage is set to a set percentage, >10% daily (or per-trip) mortality for 30 days is very damaging in all cases.

The impact of decreased egg laying rate was sizeable but not lethal to the colony and this result is similar to that found with other models. Bromenshank et al. (Bromenshenk *et al.*, 1991) (using the PC BEEPOP model) find that eggs are the least damaging of the life stages to lose and adults are the most. Similarly, Schmickl and Crailsheim (Schmickl and Crailsheim, 2007) find that reducing the ELR to 60% reduced the number of bees in the colony on day 360 down by 40% from control. In real colonies, that may experience swarming events, there may be periods of around 3 weeks with no egg laying. Even when egg production is high, as during the summer, these require no food and only a small amount of nursing, so the investment lost with an egg is minimal. Large increases of larval mortality at particular times of year (Figure 1) can have a significant impact on the colony. Individuals only spend 6 days as larvae so, as the stress was applied daily, an individual's chance of being affected during their larval period is lower for the same daily mortality than for other life stages with longer developmental periods (Table 2-3). However, the colony invests honey and pollen in feeding larvae, and also has invested care from in-hive workers; so losing larvae will represent a net loss of effort to the colony. Figure 2-6 shows the feedback

effect of high larval mortalities, at a sensitive time of year, leading to far fewer bees in the colony as a result of reduced potential worker population.

For newly emerged workers, a large amount of resources have been used to raise them to adulthood and they have not yet contributed to the colony. Depending on the state of the colony and the time of year, in-hive bees spend around 20 days before becoming a forager, meaning there is a long period over which the imposed stress can have an effect. Daily mortalities will build up quickly; a 5% daily in-hive worker mortality in August can lead to overall stage mortality of 65% and approximately 40,000 more in-hive workers dying (Figure 2-8) and further impacts from a 5% daily in-hive worker mortality (e.g. increase in larval loss and reduced food stores) are shown in Figure 2-7.

Figure 1 shows that the colony was more sensitive to in-hive worker losses than forager losses during summer, and the colony was more sensitive to forager mortality towards the end of the year. At the beginning and end of the year, the modelled colony contains mostly foragers; no new workers were emerging, there was little or no foraging taking place, and in BEEHAVE existing workers are classified as foragers once reaching a certain age. Therefore, at these times of year, impacts from forager mortality should be seen as impacts from general adult mortality. Hence, high daily adult mortalities can heavily reduce either the colony's ability to survive over winter or the colony's ability to build resources early the following year. High foraging mortality will also trigger in-hive workers to become foragers at an earlier age i.e. reduce the

age of first foraging. In reality, such precocious foragers may not be as successful as older foragers resulting in further stress to the colony (Perry *et al.*, 2015).

The colony is very sensitive to high percentages of this combined mortality throughout the year (Figure 2-1D), but, in many months not much more sensitive than the worst case daily life-stage mortality at any one time period. An explanation could be that the loss of certain life stages can lead to the loss of other life stages and hence removing e.g. both in-hive bees and larvae will not necessarily cause more damage than only removing in-hive bees, as the larvae would have died anyway due to a lack of brood care. At many time points in the year, there is one life stage in the model which the colony is highly sensitive to losing, but at other times, the colony is more sensitive to losing multiple life stages (e.g. April and September) and these dynamics need further investigation.

2.5.1 Setting the BEEHAVE simulations in the context of empirical evidence

The set of simulations described here use a precisely defined exposure period, effects on single life stages, and a stylised landscape. In reality, the heterogeneity of the cropped landscape over time and space and the relative toxicity and persistence of different pesticides in the landscape, and in the hive, may lead to a diverse range of sublethal and lethal impacts on individual bees at different life stages. The next steps in using BEEHAVE to examine more realistic scenarios will involve using detailed empirical evidence to capture those exposure routes and timeframes, for specific chemicals in precise locations, and a specific module for this is in development. There are many empirical studies showing how stressors affect individuals or, in some cases,

the colony in the short term. Long term, multi-year studies are available, yet uncommon (Cresswell, 2011; Pilling *et al.*, 2013; Godfray *et al.*, 2014), so the impact of imposed stresses over multiple years is not fully understood (Godfray *et al.*, 2014). Sandrock *et al.* (C. Sandrock *et al.*, 2014) find that 1.5 month exposure to two neonicotinoid insecticides through pollen patties starting in May leads to a 28% reduction in worker population in the following April, along with effects on brood size and food stores. Dively *et al.* (Dively *et al.*, 2015) also find effects on colony strength and overwintering success after 12 weeks (May - August) exposure to diet patties with high (20-100 ppb) levels of imidacloprid. In contrast, three studies of honeybee colony growth and survival in the field, when exposed via natural foraging on flowering crops treated with neonicotinoids, have shown no significant impact of the pesticide exposure on the colonies (for clothianidin (Cutler *et al.*, 2014; Rundlöf *et al.*, 2015) and for thiamethoxam (Pilling *et al.*, 2013)). Carreck and Ratnieks (Carreck and Ratnieks, 2015) suggest that the levels of pesticide encountered by foraging honeybees are lower in the field than used in many lab experiments. In large-scale field studies, in which the bees are placed near treated crops to forage (Pilling *et al.*, 2013; Cutler *et al.*, 2014; Rundlöf *et al.*, 2015) the bees may have lower and more variable pesticide exposure than in studies where bees are fed with an artificial feed, with pesticide added at 'field realistic' levels (Henry *et al.*, 2012; C. Sandrock *et al.*, 2014; Dively *et al.*, 2015) and this may explain why the former studies often find less damaging effects. In addition field studies offering the colony a known amount of pesticide (such as Sandrock *et al.* (C. Sandrock *et al.*, 2014) and Dively *et al.* (Dively *et al.*, 2015)) find that the impact upon the hive from the pesticide can appear sometime after exposure. The simulations presented here show how this can occur within the model: Figure 2-

6B shows how the number of larvae in the colony is affected by a 25% larval mortality in June. It is clear that there is an additional delayed impact likely due to a reduction in workers providing brood care.

These contrasts also highlight the difficulties of scaling from individual level effects to those at the colony level. The BEEHAVE model contains a large number of feedback loops, allowing in-depth investigation into how multiple stressors can disturb the colony dynamics in terms of mechanism, and which particular stresses are more damaging to the homeostasis of the colony. For example, with high forager mortality, worker bees become foragers earlier to compensate; this in turn may reduce the nursing force, increasing larval mortality (Perry *et al.*, 2015). A small increase in larval mortality can reduce the mortality of the surviving larvae from other causes such as lack of food or brood care, and reduces further losses, i.e. show a compensatory effect (Figure 2-7).

2.5.2 How do BEEHAVE simulations compare to those of other models?

The BEEHAVE model is a useful tool in the risk assessment of stressors to bees as many potential stressors can be assessed simultaneously, and testable hypotheses can be developed. Indeed EFSA have recently published a review to suggest, with further development, BEEHAVE could be the model of choice for regulatory pesticide risk assessment (Residues), 2015). Several models (Bromenshenk *et al.*, 1991; Khoury, Myerscough and Barron, 2011; Bryden *et al.*, 2013) have been used to

explore the impact of pesticides on bee colonies. However, the models of Khoury et al. (Khoury, Myerscough and Barron, 2011) and Bryden et al. (Bryden *et al.*, 2013) focus on limited portions of colony dynamics, and lack key processes required to accurately predict how a bee colony reacts to numerous stressors. One major feature lacking in these models is seasonality. We have shown that the time at which a stress is applied greatly affects the colonies' response. A stress imposed in April has little effect, while the same stress imposed in June will devastate the colony. PC BEEPOP (Bromenshenk *et al.*, 1991) is a model that includes colony dynamics similar to BEEHAVE; although BEEHAVE also includes a number of factors, such as the landscape and foraging dynamics (including the flow of energy in the form of honey stores) integrated with the colony module, which may be key to understanding how pesticides can impact the colony (Becher *et al.*, 2013). The BEEHAVE model is the only tool to date that also includes a dynamic landscape module and weather providing the potential for climate or location-specific simulations, as well as integration with the foraging and varroa & virus modules, to apply many stressors to the colony at any one time, as would be happening to real colonies in the field. Further development of BEEHAVE, with a 'pesticide module', to ensure correct implementation of exposure routes from flower, via forager, into the colony is underway. These simulations do not include differential exposure for bees of different ages or jobs within the colony. Chapter 3 presents a model exploring how in—hive distribution of pesticide-containing nectar can impact the exposure of bees and brood with the colony, and chapter 4 presents simulations using the BEEHAVE model and includes results of visitation rates to different patches in varied landscapes.

2.5.3 Can such simulations be used in future risk assessment?

To quantify the impact of a pesticide on a hive, the EFSA guidance ((Efsa), 2013b) classifies the magnitude of an effect by the % change in colony size. EFSA considers a change between 3.5% and 7% negligible; and a change larger than 35% to be large. EFSA use the model presented in Khoury et al. (Khoury, Myerscough and Barron, 2011; (Efsa), 2013b) to estimate what level of forager loss would be permissible for 'negligible' change and find that forager losses of 1.5 x control for six days; or 2 x for three days or 3 x for two days would be permissible. We have shown that effects on fecundity or brood mortality are not as impactful on the colony as adult loss, so worker loss is a conservative measure of the damage possible from a pesticide to the colony. The modelled colony has a certain capacity for compensation, which varies with the life stage affected and time of year and durations, but once the compensation threshold is exceeded the colony is likely to fail. Levels of 'background' mortality in the absence of pesticide exposure, depending on weather, forage quality and other stressors present, are likely to influence compensation capacity. Due to this compensation capacity and how it may vary with the health of the colony, a percent reduction of bees in the colony could have highly variable results on the health of the colony. Modelling, such as with BEEHAVE, could, therefore, help supplement the risk assessment procedure by teasing apart such dynamics.

LIS₅₀ and LIS₁₀ provide a tool to compare the effects of a variety of imposed stresses on the colony using BEEHAVE, treating the colony as an individual 'super' organism by using the percent chance of colony mortality as a measure of sensitivity to imposed stress. Colony failure as a result of an introduced chemical is not an acceptable

endpoint, but these indices (calculated from simulations) could be used to provide theoretical comparisons of the effects of different stressors on the colony, which may be informative in discussions of future regulatory risk assessment procedures, and protection goals. This study was designed with impacts of pesticides in mind, but impacts on the colony driven by *Varroa destructor*, related diseases, *Nosema sp.* or lack of forage sources could also be compared with LIS_x. Also, comparing LIS₁₀ and LIS₅₀ provides hypothetical evidence of which stressors, at which levels, may lead to colony tipping points, with the caveat that the tipping point will depend on the control scenario (e.g. forage availability and weather will all affect the colony's capacity for compensation). The BEEHAVE model, together with the use of LIS₅₀, allows consistent investigation into the impact of multiple stressors on the honeybee colony, and could be key for future risk evaluation.

CHAPTER 3 - Behavioral fate of pesticides: Modelling effects of honeybee behaviors on the distribution of pesticide in nectar within a hive.

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3.1 Abstract

Recently, the causes of honeybee colony losses have been intensely studied, showing that there are multiple stressors implicated in colony declines, one stressor being the exposure to pesticides. Measuring exposure of individual bees within a hive to pesticide is at least as difficult as assessing the potential exposure of foraging bees to pesticide. We present a model to explore how heterogeneity of pesticide distribution on a comb in the hive can be driven by worker behaviors, introducing the concept of “behavioral fate” of pesticides. The model contains simplified behaviors to capture the extremes of possible heterogeneity of pesticide location/deposition within the hive to compare with exposure levels estimated by averaging values across the comb. When adults feed on nectar containing the average concentration of all pesticide brought into the hive on that particular day it is likely representative of the worst case exposure scenario. However, for larvae, clustering of pesticide in the comb can lead to higher exposure levels than taking an average concentration in

some circumstances. The potential for extrapolating the model to risk assessment is discussed alongside the importance of the behavioral fate concept.

3.2 Introduction

Pesticides, particularly insecticides, have the potential to impact the honeybee colony if exposure is high enough (Johnson, 2015). The sensitivity of the colony to pesticide stress depends on the scale of the effect, the life-stage being impacted and varies over the year (Rumkee *et al.*, 2015). There has been much discussion of the real world impact of these chemicals, most recently with respect to systemic neonicotinoids (Eisenstein, 2015) and there is evidence that, at field-realistic doses, the honeybee colony may be able to compensate for pesticide effects (Genersch *et al.*, 2010; Godfray *et al.*, 2015; Henry *et al.*, 2015; Rundlöf *et al.*, 2015).

If honeybees forage on a crop that contains pesticide in its pollen or nectar, then foraging bees will come into contact with it (Krupke *et al.*, 2012). This could cause foragers to fail to return to the colony, either via direct mortality or orientation failure (Henry *et al.*, 2012). If they do return to the hive, however, they may bring pesticide into the colony where the younger, in-hive bees and brood will be exposed (Krupke *et al.*, 2012). It is difficult, but important, to estimate the level of exposure of foraging honeybees (Godfray *et al.*, 2014; Carreck and Ratnieks, 2015). It is also important to estimate exposure of bees within the hive (EFSA Panel on Plant Protection Products and their Residues (PPR), 2015; Hørig *et al.*, 2015), both brood and young adults who have not yet left the colony to forage, since it is predicted that losses of these life-stages could have a larger impact on colony health relative to the loss of the

older foraging bees (Rumkee *et al.*, 2015). The route of exposure for in-hive bees and brood is likely to be mainly via pesticides in nectar and pollen brought back by foragers (Krupke *et al.*, 2012). The exposure level will depend on the pesticide concentration in the surrounding forage, metabolism and dissipation of the pesticide along with the foraging, storage and feeding behavior of the bees (including processing into brood food by nurse bees) (Tremolada *et al.*, 2004; Bonzini *et al.*, 2011). We have developed a model that simulates what happens to the nectar when it reaches the colony, specifically focusing on how pesticide in nectar may be distributed, mixed, fed to larvae and stored in the combs of a colony. There have been many reports of pesticide residues in plants, individual bees and hive products (Francisco Sanchez-Bayo and Goka, 2014; Godfray *et al.*, 2014), however little is known about the intra-comb distribution of the pesticide (i.e. how pesticide is spread across the comb cells and how in-hive bees and brood are exposed). For example, if it is contained in nectar stored close to larvae and is therefore more likely to be fed to them, there may be a significant impact on that larval cohort. If it is processed into honey and capped, it is possible that the pesticide will dissipate before the honey is consumed and so will not have an impact (Jacobsen, Fantke and Trapp, 2015).

This potential effect of individual bees' behaviour on the distribution of pesticide in the comb, and, more generally on the exposure of bees within the hive to pesticides leads to the introduction of a novel concept, that of the "behavioral fate" of chemicals. This concept encompasses the movement of pesticides through a system directly resulting from the behaviour of the individuals as opposed to movement between compartments in the system (e.g. nectar, pollen, and wax in the beehive)

through chemical processes. This model will assess the behavioral fate of pesticide transiting from forager via comb to brood, and thus the exposure of individuals in the colony.

After nectar is brought by the foragers to the hive, it is transferred to one or more receiver bees (Kirchner and Lindauer, 1994; Hart and Ratnieks, 2001), mixing the nectar loads from multiple foragers. This nectar is then stored in comb cells by the receiver bees, and, whilst this has been reported to be a random process (Camazine, 1991), there may be patterns of storage based on global factors (such as gravity) (Johnson, 2009) or local factors (such as the contents of nearby cells, including distribution of empty cells) (Montovan *et al.*, 2013) or potentially based on the concentration of sugar in the nectar (Greco *et al.*, 2013) (although see Eyer *et al.* (Eyer *et al.*, 2015)). The stored nectar, if nectar flow into the colony is abundant, will be concentrated, turned into honey and capped for later consumption.

In principle a simple way to model the exposure of bees and brood inside the hive to pesticide would be to use the weight of pesticide brought in on a day and divide that into the total nectar volume brought into the hive on that day, giving an average daily pesticide concentration. The dose each bee then receives would then be calculated as the amount of pesticide in the volume of nectar that the bee or larva eats per day. Nectar within the hive is, however, compartmentalized into cells each potentially containing different pesticide concentrations. This heterogeneity of pesticide concentrations, arising from variability in residues in nectar from different sources

and the storage and feeding behaviors, could lead to different exposure distributions within the hive.

In order to explore how sensitive the exposure distributions of in-hive bees and brood are to different assumptions about bee behaviors, we used extremes of the behaviors mentioned above. In particular, we wanted to explore under what conditions full mixing of residues in all nectar is worst-case and under what conditions a more detailed description of exposure distribution is needed.

3.3 Model and Methods

We have developed an individual-based model (IBM) implemented in Netlogo 5.2.0 (Wilensky, 1999) to explore how the distribution of pesticide in the comb is affected by the behavior and decisions of bees. The metabolism and environmental fate of pesticides will also affect the distribution, but are not modelled here.

3.3.1 Model Description

The model is described in detail following the ODD protocol (Overview, Design concepts, Details) for the description of individual-based models (Grimm *et al.*, 2006, 2010). Selected sections of the ODD are presented here whilst the full ODD is available in appendix 3.

3.3.1.1 Purpose

The purpose of this model was to assess how different food storage and feeding behaviors of the honeybee affect the distribution of pesticide concentration in stored nectar, and explore how different distributions of pesticides affect the proportion of individuals (brood and adult bees) which will be exposed above a theoretical

threshold (set to an arbitrary level here but which could be defined based on a pesticide's toxicity). The model can then be used to assess the complexity required in introducing realistic in-hive pesticide exposure into an existing honeybee colony model (e.g. BEEHAVE (Becher *et al.*, 2014)). In particular, we set out to compare pesticide distributions as a result of the following contrasting behaviors : i) comparing multiple transfers between foragers and receivers (M) as opposed to each forager transferring nectar to a sole receiver (S); ii) comparing when receiver bees store nectar in the comb randomly (R), versus clustering (C) iii) comparing the effect of capping the nectar cells, (as a result of processing to honey) (P) versus no capping (N). We also investigate the impact of differing proportions of foragers bringing pesticide into the colony, a simplified surrogate for pesticide exposure levels in the landscape.

The model is not intended to provide accurate estimates of the absolute values of exposure or toxic effects of pesticide within the hive, rather, it is intended to explore the differences in pesticide distributions in nectar occurring from these simplified behaviors, and therefore establish the level of complexity required for a model such as BEEHAVE (Becher *et al.*, 2014; EFSA Panel on Plant Protection Products and their Residues (PPR), 2015) to ensure a conservative assessment of the risk posed by pesticides. The model simplifies feeding by the nurse bees, without modelling the production of brood food, instead having a direct transition of nectar to the larvae.

3.3.1.2 Entities, state variables and scales

3.3.1.2.1 Agents/individuals

The model contains three classes of agents: The cells of a single, one-sided hive comb, the bees and the forage patches. The cells of the hive comb are spatial units, implemented as 'patches' in NetLogo.

Each cell is characterized by the following state variables: 1) *patch_type*: patch contains nectar or a larva or is empty; 2) *nectar_volume_μl*: the current volume of nectar in the cell; 3) *pesticide_concentration_μg/L*: the concentration of pesticide in the cell, if the cell is a nectar cell; 4) *cell_nectar_concentration_μg/L*: the concentration of the sugar in the nectar contained in the cell;

A single nectar load is assumed to be 14μl, within the range reported by Huang and Seeley (2003)(Huang and Seeley, 2003)

The forage patches are characterized by the following variables: 1) *nectar_concentration_μg/L*: the concentration of sugar in the patch; 2) *field_pesticide_concentration_μg/L*: the concentration of pesticide in the patch;

There are four types of bee agents in the model: 1) foragers; 2) receivers; 3) larvae; 4) the queen. In the rest of the manuscript, 'adults' represent a combination of the foragers and receivers, who's feeding requirements are assumed to be the same for simplicity. A nectar load in the model is 14μl(Huang and Seeley, 2003) . This is the amount carried by the adult bees and is constant.

The forager bees are characterized by the following variables: 1) *pesticide_amount_μg*: the amount of pesticide carried by the forager; 2) *carrying_nectar?*: a Boolean value, true if the forager is still waiting to transfer nectar to a receiver; 3) *carrying_2nd_nectar?*: a Boolean value, true if, when multiple transfer is active, the forager is waiting to transfer the second load of nectar; 4) *nectar_sugar_concentration_μgL*: the concentration of sugar in the nectar load carried by the forager;

Receiver bees are characterized by the following variables: 1) *pesticide_weight_μg*: the amount of pesticide currently carried by the receiver; 2) *destination*: the receiver's cell of choice in which to deposit the carried nectar load; 3) *nectar_sugar_concentration_μgL*: the concentration of sugar in the nectar load carried by the receiver;

Larvae are characterized by the following variables 1) *age*: the age of the individual in days; 2) *pesticide_amount_μg*: the amount of pesticide contained in the larvae; 3) *cell_choice*: the cell the larvae will be fed from.

The queen is characterized by its location on the comb, the only role of the queen in this model is creating new brood with a realistic spatial distribution.

The spatial scale of the model is set to represent a typical comb of a National bee hive(*British Standard Bee Hive Frame Dimensions*, no date) assuming a frame of 34.1 x 20.3 cm with 4.34 cells per cm². The comb consists of a grid of square cells, 80 x

40, giving 3200 cells, a reasonable estimate of the number of worker cells on one side of a frame (Camazine, 1991).

The model runs in daily time steps with the foraging, receiving and feeding processes looped to implicitly represent hourly behaviors, (e.g. foraging, receiving, storage and feeding) and others happening once per day (processing).

3.3.1.3 Units

The model keep track of pesticide and sugar as both concentrations and mass. When dealing with volumes larger than a single bee's nectar load (such as in a nectar cell or at the forage patch) the substance is stored in the model as a concentration. When being handled by an individual, i.e. in foraging, receiving, storage and feeding, the substance is stored in the model by the mass of the substance. This facilitates the calculations required when nectar is stored or removed from a large source (cell or forage patch) and allows a practical understanding of the potential exposure of individuals to the substance within the hive (individual dose received and pesticide concentration in nectar stores). For concentrations of pesticides and sugar in the model, we use weight per volume ($\mu\text{g/L}$). The mass of a substance is measured in μg and when discussing the movement of nectar within the hive we use volume (μl). When calculating the concentration of a substance in the cell when a nectar load is added to it, the following equation is therefore used: *Concentration in cell* [$\mu\text{g} \cdot \text{l}^{-1}$] =

$$\frac{(\text{Concentration in cell} [\mu\text{g} \cdot \text{l}^{-1}] \cdot \text{volume of nectar in cell} [\mu\text{l}]) + (\text{Weight in nectar load} [\mu\text{g}])}{(\text{volume of nectar in cell} [\mu\text{l}] + \text{volume of nectar load} [\mu\text{l}])}$$

3.3.1.4 Process Overview and Scheduling

Time in the model is first split into days, at the beginning of the day, the 'daily update' procedure is called and at the end of each day nectar is processed. The main procedures of the model (Foraging, receiving storage and feeding) occur once per hour. Within these procedures, when all agents perform an action (e.g. all receivers storing nectar) they are called at random to perform this action... Procedures are performed in the following order each day:

Daily update – Occurring at the start of each day, daily count variables are reset to 0. Larvae age, and if they are above the age threshold for pupation (by default 6 days), they are removed from the model as, in reality, they pupate and feeding ceases. Eggs are then laid in empty cells to replace the lost larvae, maintaining a constant number of larvae.

Foraging – Each hour while foraging time remains, a defined percentage of foragers are assigned, at random, to one of the two patches (treated with pesticide or non-treated). They are then given a set volume of nectar from the randomly assigned patch with the relevant sugar and pesticide concentrations.

Receiving – After each foraging round, receivers take the nectar loads from foragers, chosen randomly from the population of foragers still waiting to transfer nectar. After securing a nectar load the receiver chooses a cell in which to deposit nectar,

depending on the scenario either at random or according to the sugar concentration of the nectar (clustering) and deposits the nectar load in the relevant cell.

Feeding – In the real world adult nurse bees feed the larvae, however as this is the only duty to be performed by nurse bees, in this model, nurse bees are implicit in the behavior of the larvae, and the preparation of brood food is not modelled explicitly, as pollen is not included in this model. Feeding rates in the model do not depend on the source of the nectar, although in a real hive the sugar concentration of the nectar may lead to larvae being fed different volumes (Rortais *et al.*, 2005), the sugar concentration in this model is arbitrary, and by excluding this resultant differential volume used as food we do not limit ourselves to the scenario in which the pesticide is contained in nectar with a higher sugar concentration. Conversion from weight of nectar to volume of nectar would depend on the sugar concentration of the nectar. The sugar concentration of the nectar in this model is solely used as a label to differentiate between the two nectar sources, the fact that the treated nectar has a higher sugar concentration is arbitrary. It is therefore safe to assume the volume to weight ratio of 360 μ l of nectar to 500mg (0.72 μ l/mg) of nectar as used by Schmickl and Crailsheim (Schmickl and Crailsheim, 2007). This ratio is for honey in their model, however nothing is lost in this assumption for nectar in this model as feeding rates are not based on the sugar concentration. Every hour, the closest cell to each larva that contains enough nectar for one feed is chosen. The larvae then feed on the nectar from the relevant cell. Each hour, each larva receives 0.82 μ l nectar ($163.5 \cdot 0.72 \cdot 0.0069$ - 163.5mg required to take one larva to pupation (Harbo, 2015), 0.72 – conversion to μ l, 0.0069 conversion to hours), assuming 6 days from hatching to

pupation, with the conversion of mg to μl as given above. In reality, the amount a larva is fed will change based on its age, as well as on the sugar concentration. We have kept the volume of nectar a larva eats constant across each day for simplicity. After the larvae have fed, the foragers and receivers in the model feed, removing $0.32 \mu\text{l}$ per day (Rortais *et al.*, 2005). As nurse bees are only implicit they do not feed and their exposure is not considered.

Processing – Nectar cells which are more than 95% full are ‘capped’, so they are no longer available to be fed from or deposited in, and the nectar in them is concentrated, representing the transformation to honey. In the model, this processing is simply the reduction of the volume of the nectar by 75%, maintaining the weight of pesticide in the nectar constant (based on the simplified assumption that the nectar contains 80% water (Potts *et al.*, 2004), although in reality this is variable dependent on the species and climate, and that honey contains 20% water (Frankel, Robinson and Berenbaum, 2015)). As the sugar content of the capped nectar is of no consequence in this model and there is no repercussion on the exposure of the bees to the pesticide we consider this extreme simplification of the process is reasonable, acting as a placeholder for potential expansion of the model.

3.3.2 Initialization

At the beginning of the simulation, 150 foragers 150 receivers and 400 larvae are created. In a real brood frame, a much larger proportion of the cells could be filled with larvae during the breeding season, however a single side of a single frame is modelled here providing food for the larvae and adults. Larvae are placed in the comb so there

are no more than two cells between each larva, similar to Johnson(Johnson, 2009). Initially 10% of the comb is filled with control (clean) nectar to represent that the frame has been used for brood and food storage for some time prior to a sudden pesticide-containing nectar flow. The concentration of pesticide in the nectar of the forage patch is set arbitrarily to 100 μg pesticide/L, intentionally high to ensure pesticide reaches the in-hive bees. The model was created to test the extremes of the behaviors and not the precise movement of pesticide into the comb and will therefore not provide realistic values of pesticide in the individual bees. Instead an arbitrary value allows us to focus on how the different behaviors alter how the pesticide moves through the hive and the resulting heterogeneity of pesticide residues in nectar, adults and brood to evaluate which, if any of the extremes would be the worst-case scenario in terms of risk of exceeding a given toxicity threshold. The sugar concentration of the nectar acts purely as a label as to the source of the nectar, as there is some evidence that nectar could be clustered together based on sugar concentration(Greco *et al.*, 2013). This difference in sugar concentration between nectar from the two patches serves only to test receiver bee behavior; in reality the sugar concentration will be highly dependent on species and climate.

In this model, the pesticide does not dissipate and is not metabolized in the individual bees, e.g. during feeding of larvae. Dissipation and metabolism would be highly product specific and could greatly reduce the exposure of individuals to pesticide, by leaving it out from the model we ensure a conservative estimate of the exposure and maintain generality.

3.3.3 Output

The output variables are the cumulative *pesticide doses (μg) received by larvae and adults*. These outputs were recorded daily. From these, the proportion of both adults and larvae that had received one of two hypothetical theoretical ‘threshold’ doses of pesticide (1ng and 5ng) was calculated on each day. In risk assessment this threshold would be set using an endpoint, such as the NOEL or LD₅₀ estimated in ecotoxicological studies (Campbell and Hoy, 1996).

3.3.4 Simulation scenarios

The design of the simulations was factorial: 3 behaviors, each with 2 levels: i) the storage of nectar by receivers was random (R) or clustered (C); ii) foragers transferred to single (S) or multiple (M) receivers; and iii) the nectar was processed to honey (P) or not (N). So, in total there were 8 combinations of behaviors, giving 8 “behavioural” scenarios. Alongside these, we also included two “averaged” scenarios i) The Uniform Average (U) in which the larvae received a pesticide dose calculated from the overall average concentration of pesticide in the entire comb each time they fed, i.e. the total mass of pesticide currently in the comb divided by the total volume of nectar, to show the effect of assuming full mixing of nectar from all sources of food in the hive; ii) The Daily Average (D) scenario where larvae received a pesticide dose calculated from the daily overall average concentration of pesticide in the nectar brought in on that particular day. Twenty replications of each of these ten scenarios (Table 3-1) were run, each for 30 days. Each set of simulations was run either with 50% of foragers assigned to the treated food patch or with 10% foragers assigned to the treated food patch, representing foraging i landscapes with different

proportions of food patches containing pesticide to show how a range of landscape exposures may affect the heterogeneity of exposure within the hive.

<i>Abbreviation</i>	<i>Scenario</i>
<i>CSN</i>	Clustered storage, Single transfer, No processing
<i>CSP</i>	Clustered storage, Single transfer, Processing
<i>CMN</i>	Clustered storage, Multiple transfer, No processing
<i>CMP</i>	Clustered storage, Multiple transfer, Processing
<i>RSN</i>	Random storage, Single transfer, No processing
<i>RSP</i>	Random storage, Single transfer, Processing
<i>RMN</i>	Random storage, Multiple transfer No processing
<i>RMP</i>	Random storage, Multiple transfer, Processing
<i>D</i>	Daily average pesticide concentration
<i>U</i>	Uniform average pesticide concentration

Table 3-1

Abbreviations of the ten scenarios presented.

3.3.5 Analysis

Outputs were taken directly into R from Netlogo with the "RNetLogo" library for R and analysed as follows:

To quantify the heterogeneity and spatial autocorrelation of pesticide in the cells of the frame, two indices (Gini coefficient and Moran's I) were calculated:

1. The Gini coefficient (Ceriani and Verme, 2011):

$$\frac{\sum_i \sum_j |x_i - x_j|}{2n^2 \mu}$$

a measure of inequality representing the mean distance between every pair of values divided by the mean value, giving a measure of inequality that can be compared between scenarios.

2. Moran's I (Moran, 1950):

$$\frac{N}{\sum_i \sum_j w_{ij}} \frac{\sum_i \sum_j w_{ij} (X_i - \mu)(X_j - \mu)}{\sum_i w_{ii} (X_i - \mu)^2}$$

a measure of spatial autocorrelation of the pesticide amongst the comb cells. This index involves the use of a weighting factor between each pair of values. For this analysis, two such weighting factors were used: i) the Euclidean distance between the two cells in question, and ii) an adjacency factor, 1 if the cells are adjacent and 0 otherwise. These give a value for Moran's I for both global (i) and local (ii) autocorrelation.

The distribution of pesticide doses (μg) received by the larvae and adults were plotted across all ten scenarios to see how pesticide is distributed amongst the individuals over time. For each scenario, the median dose of pesticide received by both the larvae and the adults was calculated, giving one value for the larvae and one for the adults in each of the 20 replicates.

A Kruskal-Wallis test was used to test for significant differences in the median values of pesticide doses received by both the adults and larvae, between the 10 scenarios. In total, 8 tests were run, for the pesticide doses received by the larvae and the adults, both when 50% of foragers return with pesticide and when 10% of foragers return with pesticide on days 10 and day 25 (to examine any change over time). The behavioral and averaged scenarios did not have equal variances, with lower variance in the averaged scenarios, leading to the choice of non-parametric methods. If the Kruskal-Wallis test showed significance, further investigation was carried out with post-hoc analysis using the Dunn test with a Bonferroni correction (Dunn, 2012). These pairwise analyses were used to test how, if at all, the 8 behavioral scenarios differ from the averaged scenarios (Tables 3-2 to 3-5).

Finally, the proportion of larvae and adults that had received a cumulative theoretical threshold dose of pesticide by the end of each day of the simulation was measured and plotted. This was calculated for two hypothetical 'threshold' values (1ng and 5ng), not intended to represent real world scenarios but chosen solely to further examine the impact of the modelled behavior on potential impact of pesticides within the colony, relevant to theoretical endpoints in risk assessment.

3.3.6 Verification (test of model implementation)

The model was tested to ensure it was working correctly by calculating the mass balance of the model. As nectar enters the comb, the total amount of nectar and pesticide are tracked. These are then compared against the total nectar in the comb, nectar lost through feeding, pesticide amount in the larvae, the pesticide concentration of each cell multiplied by its nectar volume in L and a variable that captures pesticide 'loss' from the model for example, when all cells are full and receivers have no place to store their nectar load.

3.4 Results

3.4.1 Heterogeneity and Spatial Autocorrelation

On day one, all scenarios lead to Gini coefficients >0.75 implying that most of the pesticide is contained in a small number of cells (Figure 3-1). This was lower in scenarios with random storage indicating reduced heterogeneity, but remained high with clustered storage.

Moran's I shows that if the receivers are placing nectar randomly, the pesticide is spaced randomly in the comb. As time moves on there is a small increase in Moran's I, as most cells contain pesticide, so there is autocorrelation on the local scale. When the receivers cluster the nectar, Moran's I is higher indicating positive spatial autocorrelation and this does not appear to change much with time.

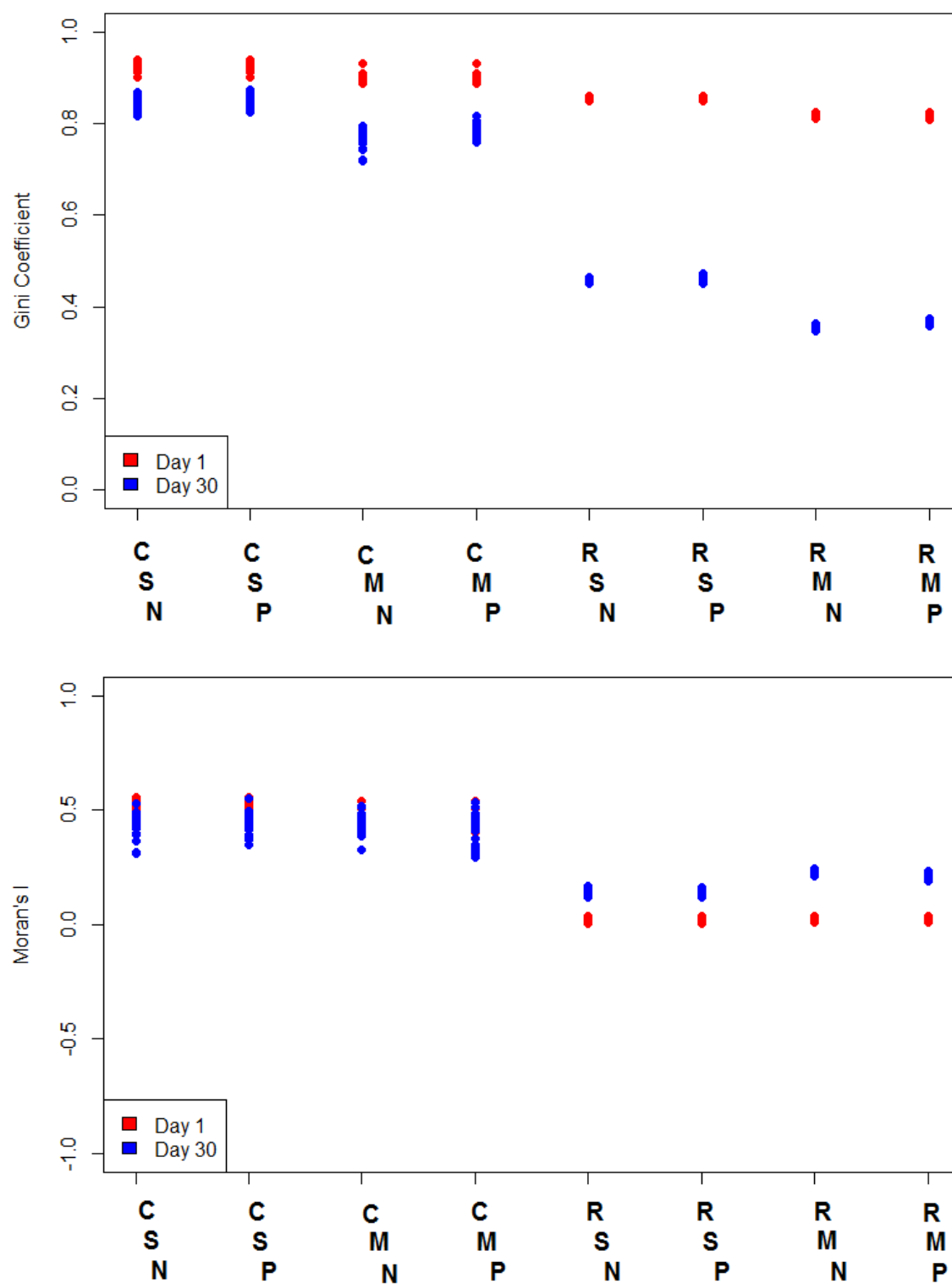


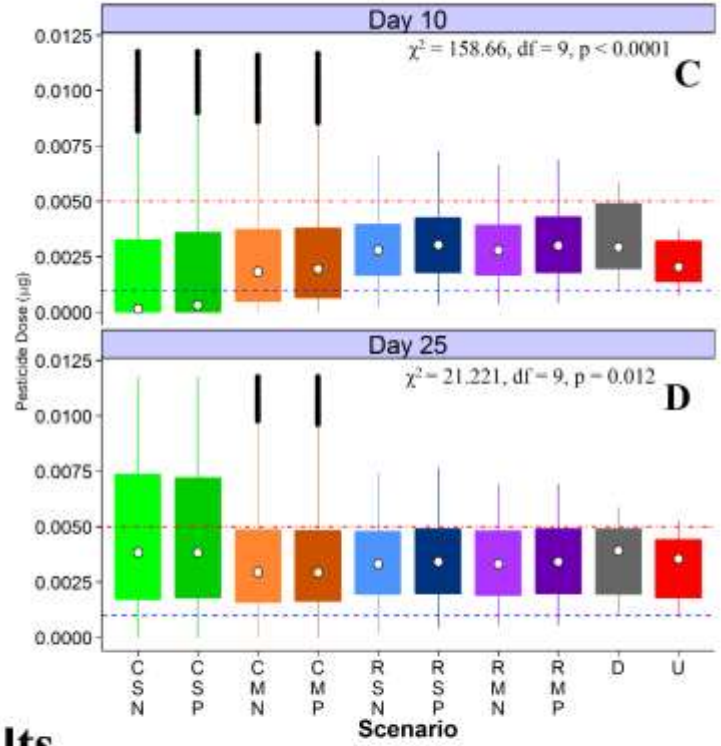
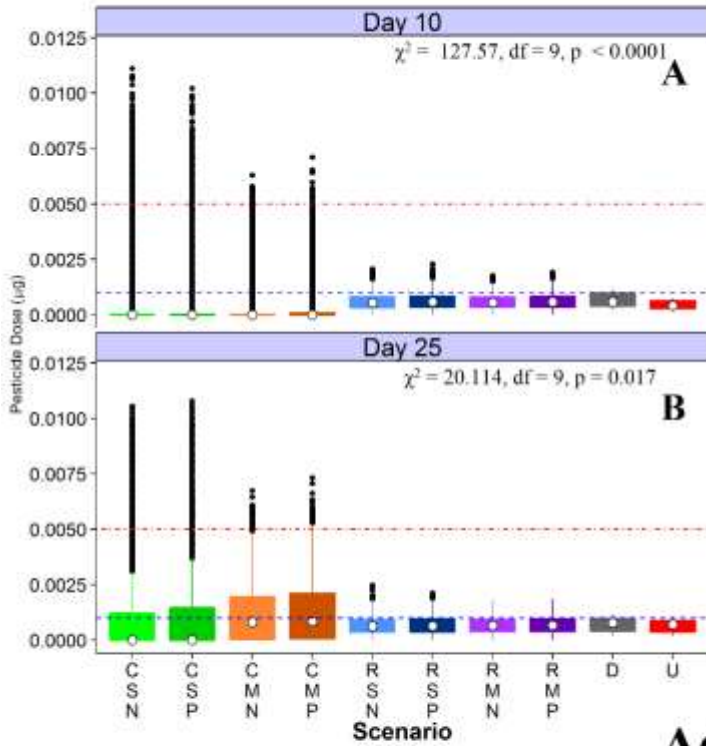
Figure 3.1

Gini coefficients and Moran's I indices on days 1 (red) and 30 (blue) for each scenario (abbreviations in Table 3.1)

Larvae

10% foragers returning with pesticide

50% foragers returning with pesticide



Adults

10% foragers returning with pesticide

50% foragers returning with pesticide

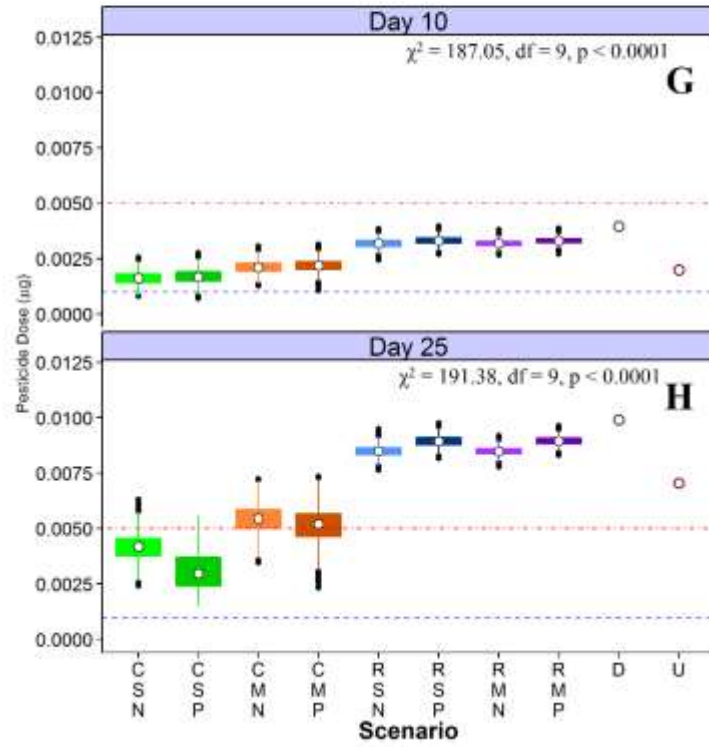
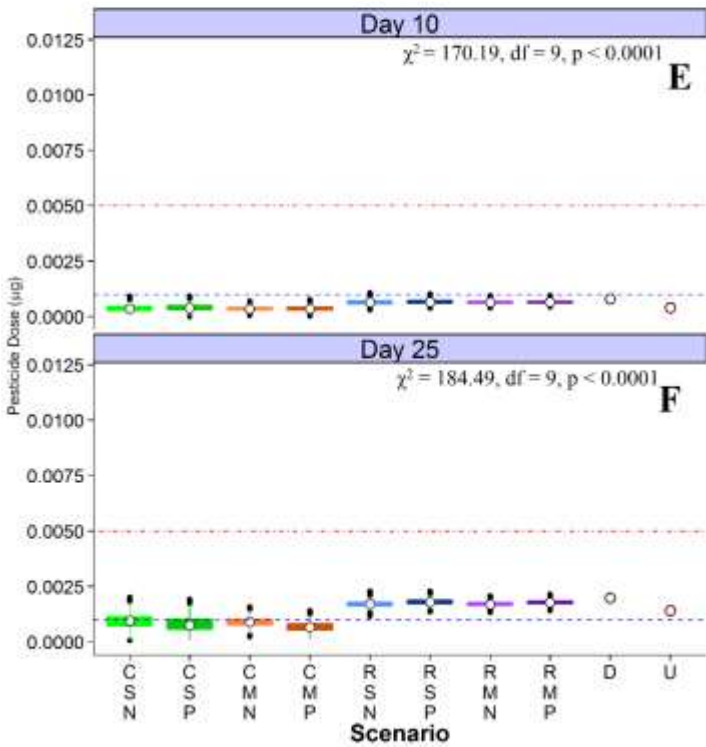


Figure 3-2

Boxplots showing the dose of pesticide in the larvae (A-D) and adults (E-H) when 10% and 50% of the foragers return with pesticide, on days 10 and 25 of each scenario (abbreviations in Table 3-1). White points show the median value of the distribution, considering all individuals across all replications. Scenarios defined by: C – Clustered storage, R – random storage, S – single transfer, M – multiple transfer, N – no processing, P – processing, D – daily average, U – uniform average. Boxes show the 25th and 75th percentiles (colors differentiate between the scenarios as in Figure 3-3), whiskers show the maximum/minimum value within 1.5x the interquartile range, any other points are shown in black. The blue and red dotted lines show the 1ng and 5ng threshold values used to explore the proportion of individuals receiving a certain pesticide dose (see Figure 3-3). With respect to the averaged scenarios: for adults, as there is no replacement of individuals, each individual gets the same pesticide dose so there is no variance (E-H). Each larva is removed from the model after 6 days (representing the start of pupation), so this, combined with the effect of the spatial positioning of any pesticide clusters, leads to a distribution of pesticide doses (A-D) in averaged scenarios.

Day 10									
	CSN	CSP	CMN	CMP	RSN	RSP	RMN	RMP	D
CSP	0.623 -								
CMN	0.847 -	0.224 -							
CMP	0.639 -	0.016 -	0.208 -						
RSN	5.297 ***	4.674 ***	4.45 ***	4.658 ***					
RSP	6.469 ***	5.846 ***	5.622 ***	5.83 ***	1.172 -				
RMN	5.581 ***	4.958 ***	4.734 ***	4.942 ***	0.284 -	0.888 -			
RMP	6.906 ***	6.283 ***	6.059 ***	6.267 ***	1.609 -	0.437 -	1.325 -		
D	8.012 ***	7.389 ***	7.165 ***	7.373 ***	2.715 -	1.543 -	2.431 -	1.106 -	
U	3.68 *	3.057 -	2.833 -	3.04 -	1.617 -	2.789 -	1.901 -	3.226 -	4.333 ***
Day 25									
	CSN	CSP	CMN	CMP	RSN	RSP	RMN	RMP	D
CSP	0.423 -								
CMN	2.778 -	2.355 -							
CMP	3.327 *	2.904 -	0.549 -						
RSN	1.762 -	1.339 -	1.016 -	1.565 -					
RSP	2.073 -	1.65 -	0.705 -	1.254 -	0.311 -				
RMN	2.175 -	1.751 -	0.604 -	1.153 -	0.413 -	0.101 -			
RMP	2.688 -	2.265 -	0.09 -	0.639 -	0.926 -	0.615 -	0.514 -		
D	2.847 -	2.423 -	0.068 -	0.481 -	1.085 -	0.773 -	0.672 -	0.158 -	
U	1.841 -	1.418 -	0.937 -	1.486 -	0.079 -	0.232 -	0.333 -	0.847 -	1.005 -

Table 3-2

Detailed results from post-hoc pairwise analysis of distribution of median pesticide doses in larvae when 10% of foragers return with pesticide.

*Z values of the Dunn post-hoc test are presented along with significance. $p\text{-value} < 0.05 = *$, $< 0.01 = **$, $< 0.001 = ***$.*

Day 10									
	CSN	CSP	CMN	CMP	RSN	RSP	RMN	RMP	D
CSP	0.148 -								
CMN	2.024 -	1.877 -							
CMP	2.355 -	2.207 -	0.331 -						
RSN	5.215 ***	5.067 ***	3.191 -	2.86 -					
RSP	7.463 ***	7.316 ***	5.439 ***	5.108 ***	2.248 -				
RMN	5.207 ***	5.059 ***	3.183 -	2.852 -	0.008 -	2.256 -			
RMP	7.22 ***	7.073 ***	5.196 ***	4.865 ***	2.005 -	0.243 -	2.013 -		
D	7.881 ***	7.734 ***	5.857 ***	5.526 ***	2.666 -	0.418 -	2.674 -	0.661 -	
U	2.781 -	2.633 -	0.757 -	0.426 -	2.434 -	4.682 ***	2.426 -	4.439 ***	5.1 ***
Day 25									
	CSN	CSP	CMN	CMP	RSN	RSP	RMN	RMP	D
CSP	0.429 -								
CMN	2.505 -	2.934 -							
CMP	2.642 -	3.071 -	0.137 -						
RSN	1.12 -	1.549 -	1.385 -	1.522 -					
RSP	0.09 -	0.519 -	2.415 -	2.551 -	1.03 -				
RMN	1 -	1.429 -	1.505 -	1.642 -	0.12 -	0.91 -			
RMP	0.372 -	0.8 -	2.134 -	2.27 -	0.749 -	0.281 -	0.628 -		
D	0.309 -	0.738 -	2.196 -	2.333 -	0.811 -	0.219 -	0.691 -	0.063 -	
U	1.953 -	2.382 -	0.552 -	0.688 -	0.833 -	1.863 -	0.953 -	1.582 -	1.645 -

Table 3-3

Detailed results from post-hoc pairwise analysis of distribution of median pesticide doses in larvae when 10% of foragers return with pesticide.

*Z values of the Dunn post-hoc test are presented along with significance. $p\text{-value} < 0.05 = *$, $< 0.01 = **$, $< 0.001 = ***$*

Day 10									
	CSN	CSP	CMN	CMP	RSN	RSP	RMN	RMP	D
CSP	0.287 -								
CMN	0.647 -	0.934 -							
CMP	0.317 -	0.604 -	0.331 -						
RSN	4.3 ***	4.013 **	4.947 ***	4.617 ***					
RSP	5.824 ***	5.537 ***	6.472 ***	6.141 ***	1.524 -				
RMN	4.002 **	3.715 **	4.65 ***	4.319 ***	0.298 -	1.822 -			
RMP	5.794 ***	5.507 ***	6.442 ***	6.111 ***	1.494 -	0.03 -	1.792 -		
D	7.712 ***	7.425 ***	8.359 ***	8.029 ***	3.412 *	1.888 -	3.71 **	1.918 -	
U	0.992 -	0.705 -	1.639 -	1.309 -	3.308 *	4.833 ***	3.01 -	4.802 ***	6.72 ***
Day 25									
	CSN	CSP	CMN	CMP	RSN	RSP	RMN	RMP	D
CSP	0.691 -								
CMN	0.249 -	0.443 -							
CMP	1.377 -	0.686 -	1.128 -						
RSN	3.939 **	4.63 ***	4.188 **	5.316 ***					
RSP	6.1 ***	6.791 ***	6.349 ***	7.477 ***	2.161 -				
RMN	3.729 **	4.42 ***	3.977 **	5.106 ***	0.21 -	2.371 -			
RMP	5.77 ***	6.461 ***	6.018 ***	7.146 ***	1.83 -	0.331 -	2.041 -		
D	7.616 ***	8.307 ***	7.865 ***	8.993 ***	3.677 *	1.516 -	3.887 **	1.847 -	
U	2.153 -	2.844 -	2.401 -	3.529 *	1.787 -	3.947 **	1.576 -	3.617 *	5.464 ***

Table 3.4

Detailed results from post-hoc pairwise analysis of distribution of median pesticide doses in adults when 10% of foragers return with pesticide.

*Z values of the Dunn post-hoc test are presented along with significance. p -value < 0.05 = *, < 0.01 = **, < 0.001 = ***.*

Day 10									
	CSN	CSP	CMN	CMP	RSN	RSP	RMN	RMP	D
CSP	0.385 -								
CMN	2.737 -	2.352 -							
CMP	3.204 -	2.819 -	0.467 -						
RSN	5.543 ***	5.158 ***	2.806 -	2.338 -					
RSP	7.679 ***	7.294 ***	4.942 ***	4.475 ***	2.136 -				
RMN	5.513 ***	5.128 ***	2.776 -	2.308 -	0.03 -	2.166 -			
RMP	7.698 ***	7.313 ***	4.961 ***	4.494 ***	2.155 -	0.019 -	2.185 -		
D	9.34 ***	8.955 ***	6.603 ***	6.136 ***	3.797 **	1.661 -	3.827 **	1.642 -	
U	2.128 -	1.743 -	0.609 -	1.076 -	3.415 *	5.551 ***	3.385 *	5.57 ***	7.212 ***
Day 25									
	CSN	CSP	CMN	CMP	RSN	RSP	RMN	RMP	D
CSP	0.948 -								
CMN	1.661 -	2.609 -							
CMP	1.243 -	2.191 -	0.418 -						
RSN	4.912 ***	5.86 ***	3.251 -	3.669 *					
RSP	7.023 ***	7.971 ***	5.363 ***	5.78 ***	2.112 -				
RMN	4.808 ***	5.756 ***	3.147 -	3.565 *	0.104 -	2.215 -			
RMP	7.067 ***	8.015 ***	5.406 ***	5.824 ***	2.155 -	0.044 -	2.259 -		
D	8.684 ***	9.632 ***	7.023 ***	7.441 ***	3.773 **	1.661 -	3.876 **	1.617 -	
U	3.221 -	4.169 **	1.56 -	1.978 -	1.691 -	3.803 **	1.587 -	3.846 **	5.464 ***

Table 3-5

Detailed results from post-hoc pairwise analysis of distribution of median pesticide doses in adults when 50% of foragers return with pesticide.

*Z values of the Dunn post-hoc test are presented along with significance. p -value $< 0.05 = *$, $< 0.01 = **$, $< 0.001 = ***$.*

3.4.2 Effect of behavior on distribution of pesticide doses

Kruskal-Wallis tests showed there were significant differences between the scenarios in the median pesticide doses received by larvae and by adults, both when 10% and 50% of the foragers return to the colony with pesticide, on both days 10 and 25 of the simulations i.e. for all eight comparisons, prompting post-hoc analyses (presented in Tables 3.2-3.5). Patterns of results are discussed for larvae and adults separately below.

3.4.2.1 Larvae

When 10% of foragers return with pesticide, the median doses received by larvae were low after 10 and 25 days of the simulations (Figure 3-2A, B), for all scenarios. As expected they were higher when 50% of foragers return with pesticide (Figure 3-2C, D). In all comparisons (Figure 3-2A-D), the *variation* in dose received by larvae was highest for the clustered scenarios.

Results of the pairwise analyses showed similar (although not identical) patterns for both 10% (Table 3-2) and 50% of foragers (Table 3-3) returning with pesticide: On Day 10, the daily average scenario led to a median pesticide dose higher than all scenarios, other than scenario RMP and was significantly different ($P < 0.001$ in all cases) to the scenarios with clustered storage (which had the lowest medians) and to the uniform average scenario. The median pesticide doses received in clustered scenarios were also significantly lower than the random scenarios. The uniform

average scenario varied in its position in ranking of medians. On Day 25, there were no significant differences between pesticide doses received in the two averaged scenarios and any of the eight behavioral scenarios. Landscape exposure (10% or 50% of foragers returning with pesticide) appeared to have more effect on average exposure of larvae, than the modelled behavioral scenarios (Figure 3-1A-D) although this was not compared statistically.

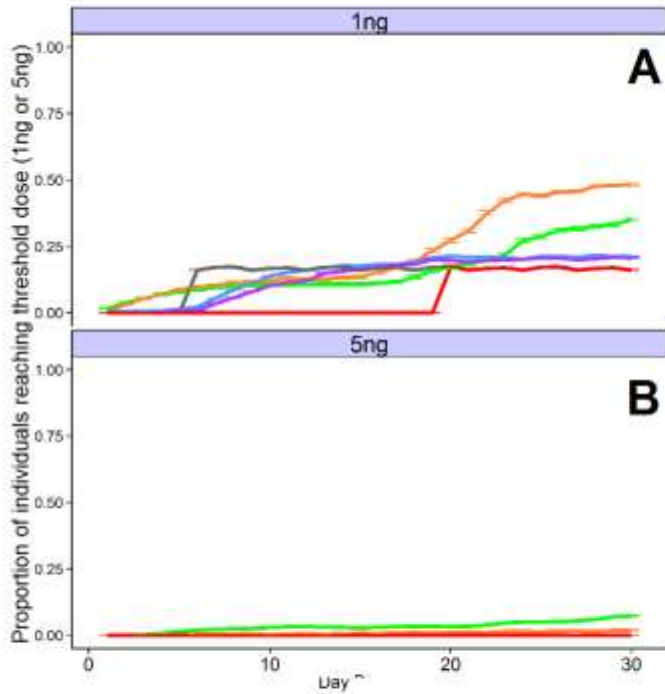
3.4.2.2 Adults

Median doses received by adults showed similar patterns (Figure 3-1E-H). Although the variation in dosage to adults within a scenario was much less than for larval doses, it was still greater as a result of clustering behavior.

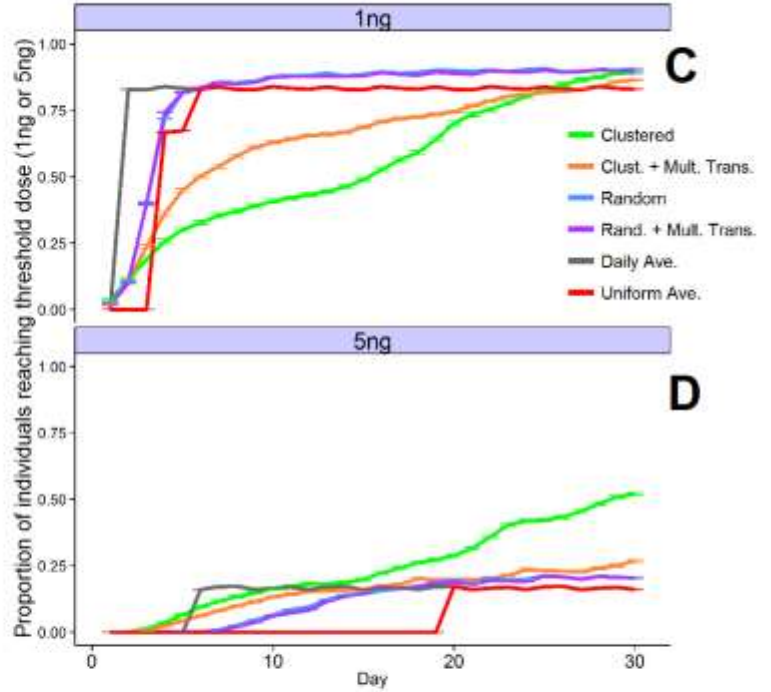
For 10% and 50% of foragers returning with pesticide, the patterns in the pairwise analyses results were similar for Day 10 and Day 25 (Table 3-4 & Table 3-5): again the daily average scenario resulted in the highest median dosage to adults and this was significantly different ($P < 0.001$ in all cases) to the scenarios with clustered storage (which had the lowest medians) and to the uniform average scenario, but also to the scenarios with random storage and no processing (RSN, RMN). As with the larvae, the clustered scenarios resulted in significantly lower median doses to adults, than the random scenarios. The uniform average scenario also often resulted in a significantly lower dose to adults than some of the random scenarios. Overall landscape exposure (10 or 50%) appeared to have greater impact than the different behavior scenarios (Figure 3-2E-H).

Larvae

10% foragers returning with pesticide

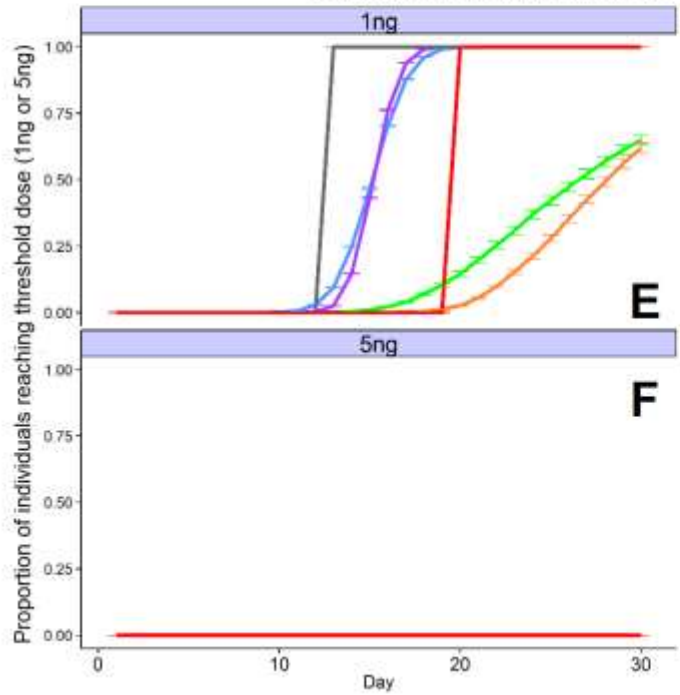


50% foragers returning with pesticide



Adults

10% foragers returning with pesticide



50% foragers returning with pesticide

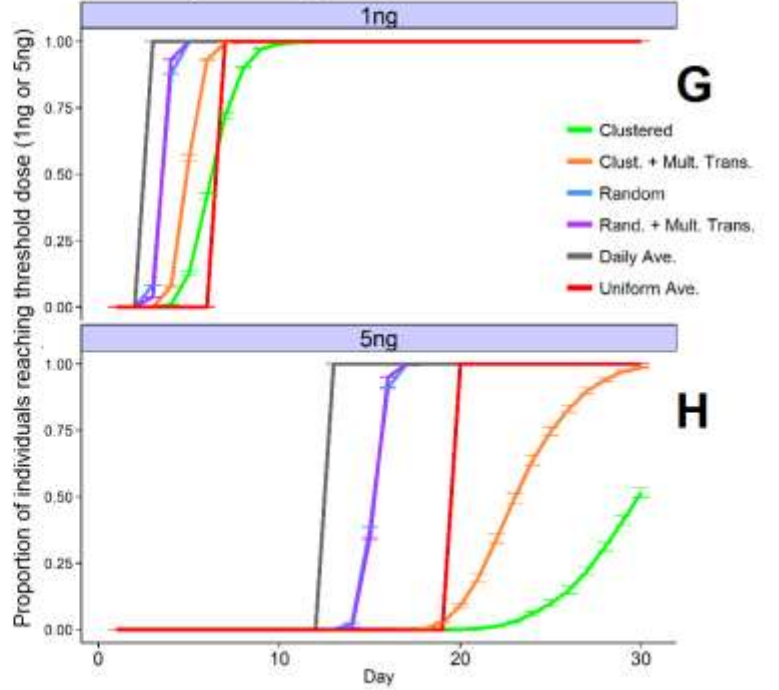


Figure 3-3

The mean (\pm standard error) proportion of larvae (A-D) and adults (E-H) that received two “threshold” levels of pesticide over the course of 30 days (means of 20 replicates). The two “averaged” scenarios (Daily average pesticide concentration and Uniform average pesticide concentration) are shown, along with the four scenarios without nectar processing.

3.4.3 Effect of behavior on the proportion of individuals at risk

3.4.3.1 Proportions of larvae at risk

When 10% of the foragers returned to the colony carrying pesticide, until around day 19, in all scenarios, the proportion of larvae receiving the 1ng theoretical threshold dose remained below 0.25 (Figure 3-3A). After day 19, scenarios in which receivers clustered nectar had a higher proportion of larvae receiving the 1ng dose than scenarios with random storage or averaged pesticide concentrations in the food, with the addition of multiple transfer further increasing the proportion (Figure 3-3A). For the 5ng threshold, only the scenarios with clustered storage led to any of the larvae reaching the threshold (Figure 3-3B) with around 10% of bees reaching the threshold by day 30.

When 50% of the foragers returned to the colony carrying pesticide, scenarios in which the receivers cluster nectar led to the proportion of larvae reaching the 1ng threshold to rise more slowly than in the other scenarios (Figure 3-3C) as only larvae close to the pesticide cluster receive any pesticide dose. The addition of multiple transfers alongside clustered placement increases this proportion. When considering the 5ng threshold (Figure 3-3D), after day 12, the scenario in which the receivers cluster nectar lead to a higher proportion of larvae reaching the threshold than scenarios with random placement and the two averaging scenarios. When multiple transfers are also occurring alongside clustered storage, the proportion of larvae receiving the 5ng threshold remains lower and closer to the average scenarios.

3.4.3.2 Proportions of adults at risk

A higher proportion of adults reach both threshold doses in the scenarios where adults feed from nectar with the daily average pesticide concentration (Figure 3-3E-H) than any other scenario, regardless of the proportion of foragers returning with pesticide. In the uniform average scenario, regardless of the proportion of foragers returning to the colony with pesticide, it takes longer for 100% of the adults to reach either threshold dose than the daily average or scenarios in which the receivers place nectar randomly. Scenarios in which receivers are clustering nectar lead to a lower proportion of adults reaching the threshold doses than when the receivers are storing randomly. In these scenarios, the pesticide is stored in fewer cells, as the adults pick cells at random, it is less likely that they feed from cells containing

pesticide. When only 10% of foragers return to the colony with pesticide, no adults reach the 5ng threshold (Figure 3-3F).

3.5 Discussion

The results from the model presented show that the three behaviors we simulated can lead to significantly different distributions of pesticide doses received by both the larvae and in-hive worker bees (Figure 3-2, 3-2). The results also show that, in most cases, assuming each larva or adult feeds on the daily average pesticide concentration (total weight of pesticide brought in on a particular day / total nectar volume brought in) led to higher median doses received by both the larvae and the adult bees (Figure 3-2; Tables 3-2...3-5), although effects of different behaviors were seen on the *distribution* of those doses amongst individuals (Figure 3-2), and on the likelihood and rate at which larvae or adults reach theoretical threshold doses (Figure 3-3). In particular, the way in which receivers choose to store nectar in the comb (random or not) appears to be much more impactful than whether or not multiple transfer between receivers and foragers takes place, or if some pesticide is removed from the system (capped) in the process of turning the nectar to honey.

The heterogeneity and spatial autocorrelation of pesticide in the cells of the comb (captured by the Gini coefficient and Moran's I respectively, Figure 3-1) show that on Day 1, regardless of the scenario, the pesticide is only contained in a few of the cells. On Day 30, those scenarios with random storage show that the pesticide is more evenly distributed across the cells, however with clustered storage the pesticide remained in fewer cells, which showed some positive autocorrelation. The

distribution of pesticide doses received by the individuals (Figure 3-2) shows, as expected, that when the receiver bees cluster the pesticide-containing nectar, the medians are lower for larvae and adults than when the pesticide-containing nectar is placed randomly. However, for larvae, there is a broader distribution in clustered storage scenarios such that some larvae receive a much higher maximal dose (Figure 3-2A-D) and more larvae may reach a critical threshold depending on the level of exposure in the landscape (Figure 3-3A-D). In the model, the larvae get food from the cell closest to them with enough nectar to facilitate a single feed. If the pesticide-containing nectar is clustered close to the larvae, those larvae will only be fed on this nectar, leading to the high maximum dose received. In situations where a smaller proportion of the foragers are bringing pesticide into the colony, if there is a cluster of pesticide near the larvae, then some larvae will still be receiving large amounts of pesticide. In Figure 3-3A & B, this is observable as a higher proportion of the larvae received doses meeting the threshold values in the scenarios with just clustering (CSN) and that with clustering and multiple transfer (CMN) than the daily average scenario.

In contrast adults feed randomly from the comb in the model so, even if pesticide-containing nectar is clustered in the comb, over a number of feeds the individual adults will receive a mixture of doses and thus lower maximum doses (Figure 3-2E-H). In the case of the adult bees, assuming they feed on nectar containing the daily average pesticide concentration gives the most conservative estimate of exposure for all scenarios (Figure 3-2E-H). In Chapter 2 (Rumke *et al.*, 2015) I show that the colony

is highly sensitive to the loss of in-hive adult workers and, as such, it is useful to know that we can assume averaging as the most conservative estimate.

Based on this model, however, taking the uniform average of total pesticide in the comb across the total nectar volume in the comb does not in most cases lead to a conservative estimate of the individual level exposure for larvae or adults. In practical terms, these results provide an argument that sampling nectar from random cells across the comb to estimate residue levels (equivalent to U) would not give a conservative estimate of risk. Sampling nectar coming into the colony on a daily basis (equivalent to D) (for example sampling honey stomachs from returning foragers) may be more appropriate in the majority of cases.

The results highlight the importance of the behavioral fate of a pesticide. We have shown that the behaviors of individual bees could influence the movement of pesticide throughout the hive system, and should be considered together with the chemical properties of the pesticide in question influencing the movement between compartments (e.g. nectar, wax, bees etc.). In fact for the same amount of pesticide entering the hive, the behavioral movement of pesticides can have a considerable impact on the resultant exposure of individuals to the pesticide, and, although a daily average is a more conservative estimate of pesticide exposure, the behavioral fate of the pesticide may need to be considered in some circumstances when attempting to assess realistic exposure. However it should be noted that whilst the model was not designed to compare the effects of in-hive behaviors with the effects of external exposure levels, the proportion of foragers bringing contaminated nectar into the hive

(set at 10% or 50%) did have considerably more impact on pesticide dosage to larvae and in-hive adults than in-hive behaviors, although this is not surprising given the five-fold difference in simulated landscape exposure.

The spatial clustering in the model is extreme, with all pesticide-containing cells next to each other. If this extreme clustering of pesticide containing nectar is no worse than full mixing in terms of pesticide exposure, then it follows that less extreme clustering would also be no worse. However, for larvae, we have shown that extreme mixing *can* lead to a higher proportion of larvae receiving some pesticide doses in some circumstances (Figure 3-3A-D). There is some empirical evidence that clustering of nectars of similar sugar concentrations can occur (Greco *et al.*, 2013), although Eyer *et al.* (Eyer *et al.*, 2015) find clustering of nectar of similar sugar concentrations only occasionally and that this clustering effect is not found after around 3 days. We assume a simplified larval feeding process, however, in the real hive, a nurse will prepare brood food and this may result in increased mixing. However, as this is clustering based on sugar, the resultant pesticide distribution from this clustering behavior would be unknown. The model could, if necessary, be modified to also include the movement of pollen in to the model, and to make nurse bees explicit, they can then prepare brood food from pollen and nectar depending on the age of the larvae.

The model also only considers a single pesticide in one of only two forage patches, however in the real landscape there will be many more sources of nectar and, depending on the landscape, a number of sources of pesticides. An abundance of

sources of nectar and pesticide is likely to increase the mixing of pesticide within the comb as, even if receivers sort nectar by sugar concentration, there may be nectar sources with similar sugar concentrations and yet varying pesticide concentrations and vice versa. Along with multiple transfers, nectar will likely be mixed within the hive by in-hive workers removing nectar from one cell and moving it into another, further reducing the heterogeneity of pesticide concentration across the comb cells. The model results imply that assuming the larvae are fed pesticide with an averaged pesticide concentration, or from nectar that is well mixed is not, in all cases, the worst case scenario however this will depend on the levels of pesticide in the landscape. As the model is intended to be extreme, more detailed investigation would be needed to assess exactly what level of pesticide clustering is realistic and the complexity of in-hive pesticide distribution necessary to obtain a worst-case exposure estimate for the larvae, this further investigation would, in part, consist of a rigorous sensitivity analysis. When considering the exposure of the larvae to pesticides, the model results highlight the importance of knowing the prevalence of the specific pesticides in the landscape. If there is little pesticide in the landscape (here simulated by only 10% of foragers returning with pesticide), and if the pesticide in question is highly toxic to the larvae (here simulated as a 1ng threshold, Figure 3-3A), then the clustering of nectar in the colony may have a significant effect on the resultant impact of the pesticide on both individuals, and therefore potentially on the colony (Chapter 2 and (Rumkee *et al.*, 2015). Similarly, if the pesticide is prevalent (e.g. present in 50% of the forage sources) then Figure 3-3C & D imply that assuming an average dose is fed to the larvae is worst-case if the threshold dose required for an effect is low, as all larvae are likely to reach the threshold, but this is not the case for less toxic pesticides with higher

thresholds (here simulated as 5ng). Chapter 4 presents results of simulations from the BEEHAVE model in various landscapes and shows visitation rates to food sources in the landscape, this could be used in conjunction with these results to estimate the proportion of foragers returning with pesticide-contaminated nectar. The European Food Safety Authority (EFSA) recently reviewed the BEEHAVE model (Becher *et al.*, 2014) and highlighted the need for a pesticide module (EFSA Panel on Plant Protection Products and their Residues (PPR), 2015). If necessary the model presented here could be incorporated into such a module, for the situations in which assuming an average, fully mixed pesticide concentration is not the most conservative estimate for exposure via nectar (e.g. Fig 3-3A: high toxicity pesticide affecting the larvae). We suggest that the behavioral fate of pesticides could be a valuable route for empirical research, as we have shown that, in the case of honeybees it can lead to a significant change in the exposure of individuals within the colony to pesticides, it is likely that this will be the case in other areas of ecotoxicology.

Chapter 4 – Modelling the impact of foraging mortality and a sublethal impact to foraging on a honeybee colony in two landscapes

4.1 Abstract

It is known that pesticides have the potential to impact the honeybee colony. One of the main routes of exposure is through contaminated pollen and nectar. While the foraging bees are collecting the food, depending on the concentration of pesticide in the nectar, they may die, or in some way become lost to the colony before they are able to return. There may also be a sublethal effect of pesticides on foraging behaviour, reducing the efficiency of foragers. In this chapter, I present results of simulations using the BEEHAVE model in which an increased mortality is applied to the foraging bees during the foraging trip both as a chronic 30-day exposure of 5% mortality pre trip in April, June or August and as an acute exposure of a single day of 100% foraging mortality on the first day of each month. A sublethal impact was imposed as a reduction in the time available for the foragers to forage. These simulations took place in two landscapes, one simple landscape consisting of two

patches (one treated and one untreated) and a complex landscape consisting of 115 patches, based on a real landscape. The results show that the landscape affects the resultant impact of a pesticide exposure (both lethal, acute and chronic, and sublethal). If there is untreated forage closer to the colony than treated forage, the impact of the pesticide exposure is lessened. The timing of an exposure is also important. These results imply that providing a beneficial landscape could mitigate any potential negative impacts of pesticides.

4.2 Introduction

Along with many other species of pollinator, the honeybee faces a number of stressors (Potts *et al.*, 2010; vanEngelsdorp and Meixner, 2010) in its environment, some natural and some anthropogenic. These include changes to the landscape due to agriculture, such as a reduction in the amount of forage due to removal of wild flowers or extensive monoculture (Naug, 2009); the use of pesticides on crops (Johnson, 2015), leading to exposure of the foraging honeybees either from direct contact or orally through consumption of the nectar, and then exposure of in-hive bees through the contaminated brought back to the colony (Krupke *et al.*, 2012); presence of parasites and pathogens such as *Varroa destructor* (Rosenkranz, Aumeier and Ziegelmann, 2010)(Boecking and Genersch, 2008; Genersch *et al.*, 2010), *Nosema spp.*

(Charbonneau *et al.*, 2016) and various viruses. This particular study is intended to focus on the impact of pesticides.

During any one year, depending on the landscape in which it is located, a honeybee colony is likely to come into contact with a number of pesticides through a number of routes (Krupke *et al.*, 2012). For a large proportion of the year, from late spring through into the autumn in temperate climates, bees will be exploring the landscape to locate and collect food, in the form of nectar and pollen from plants. In agricultural landscapes especially, these plants are likely to have been treated with a number of pesticides. If this treatment was as a spray then any bees foraging at the time of spraying (or soon after) will come into contact with the pesticides. A number of pesticides will harm or kill the honeybees on contact (Bailey *et al.*, 2005) if the residue remains on the surface of the plant. If the pesticide is systemic in nature, then it is likely to move into nectar and pollen making it available to the foraging bees for an extended period (Stoner and Eitzer, 2012).

It is known that a number of pesticides have the ability to impact individual honeybees, depending on the level of exposure. As well as an acute toxic effect increasing the mortality of the individuals, there have also been numerous sublethal effects shown (Desneux, Decourtye and Delpuech, 2007). These sublethal effects include change in

egg and larval weight (Dai *et al.*, 2010), reduced lifespan (Wu, Anelli and Sheppard, 2011), effects on learning and memory (Aliouane *et al.*, 2009)(Decourtye *et al.*, 2004) and reduced locomotor abilities (Charreton *et al.*, 2015) amongst others. Many of these sublethal effects will reduce the foraging effort of the hive.

There have been a number of attempts to model the impact of foraging mortality and sublethal effects of pesticide on the honeybee colony. Henry *et al.* (Henry *et al.*, 2012) found that a “field-realistic sublethal dose of thiamethoxam” (1.34ng in 20µl of sucrose solution; but see Cresswell and Thompson 2012 (Cresswell and Thompson, 2012)) is sufficient to result in a mortality double that of the natural foraging mortality, presumed to be due to orientation failure leading to the individual never returning to the colony, which for all practical purposes is the same as a lethal dose having been consumed since the bee is lost from the colony either way. They then use the Khoury model (Khoury, Myerscough and Barron, 2011) to show that this increase in mortality could seriously impact the colony by significantly reducing the colony population (Henry *et al.*, 2012). Bryden *et al.* (Bryden *et al.*, 2013) present a model to study the impact of an arbitrary sublethal effect on a bumblebee colony, and, combining this with empirical work found that sublethal effects from a pesticide can, once again, significantly impact the colony dynamics. Thompson *et al.* (Thompson *et al.*, 2007) use a model to show that premature aging can be extremely harmful to the colony, which has been shown empirically by Perry *et al.* (Perry *et al.*, 2015). These studies, however, use relatively simple models which, whilst well suited to exploring a general impact to hive dynamics

or focussing on one or a small number of mechanisms, are unable to fully explore the impact of a sublethal effect with all the feedback loops and compensatory mechanisms present in the colony which may exacerbate or reduce the damage a sublethal effect could cause.

The BEEHAVE model (Becher *et al.*, 2014) is a more holistic model, incorporating a large number of colony processes and realistic foraging dynamics. (For details, refer to chapter 1 pg. 46). The BEEHAVE model contains a number of feedback loops and compensatory mechanisms within and between the modules which will give a more mechanistic view of the potential impacts of foraging mortality and sublethal impacts on foraging. Thorbek *et al.* (2016a) (Thorbek *et al.*, 2016)¹ use the BEEHAVE model to examine the mitigating effect of an untreated patch of forage in the landscape alongside a pesticide treated patch at varying distance, until both patches are 1km from the colony, and show that the addition of an untreated patch reduces the impact of a treated patch on the colony. They also show that in general as forage is moved away from the colony, increased foraging mortality has a greater impact on the colony. Thorbek *et al.* (2016b) (Thorbek, Campbell and Thompson, 2016) look at the impact of various sublethal impacts on the BEEHAVE colony, finding that the impact of the

¹ Please note, the work in this chapter was done in 2013, before Thorbek *et al* 2016a.

effects tested was different depending on the crop type in the landscape, and that a beneficial landscape mitigated these impacts.

In this study, we use the BEEHAVE model, to explore the impact of increased foraging mortality and sublethal effects on foraging behaviour on the honeybee colony in two landscapes, one simple (a theoretical landscape consisting of two patches) and one complex (based on a real world map). These landscapes will contain some patches that are treated with a theoretical pesticide, and some that are not. This presents the colony with a simplified 'exposure landscape' to investigate how the landscape configuration (distance to patches of varying quality) affects the impact on the colony. The direct foraging mortality is to be applied in two ways, to represent a chronic exposure, for example to a systemic pesticide applied as a seed coating, or a short, highly toxic exposure, for example to a pesticide foliar spray event. Sublethal effects of pesticides on foraging behaviour can be highly varied, so a simplified substitute of a general reduction in foraging, by reducing the number of hours each day in which foraging can take place, will be used as a simplified alternative.

4.3 Model and Methods

4.3.1 Model parameterisation

The model was parameterised as outlined in Becher et al. (Becher *et al.*, 2014) and also Rumkee et al. (Rumkee *et al.*, 2015) (Chapter 2), starting on January 1st with 10000 bees in the colony. The colony in all scenarios was free from varroa and

disease, as the aim of the study was to focus in detail on the differential impact of foraging mortality and sublethal effects on foraging at different times and in different landscapes. Weather in the model was parameterised using maximum daily temperatures and the number of sunlight hours from data collected at the meteorological station at Rothamsted Research, Hertfordshire, U.K in 2009. At the end of each year (on December 31st) any colonies with 4000 bees or less was considered dead as it is estimated that this is a threshold number of winter bees, below which the colony will not survive winter(Becher *et al.*, 2014).

4.3.2 Landscape

The BEEHAVE model allows the landscape to be defined dynamically by the user. Each of the food patches is defined by: distance to the colony (m), area (m²), and values for the handling time for nectar and pollen (s). Additionally, for each day of the year, the nectar quality (sucrose concentration (mol/l)), nectar quantity (L), and pollen quantity (kg) can be defined via an input file. The quantity of both pollen and nectar can be set to follow a seasonal curve, based on the model from Schmickl and Crailsheim(Schmickl and Crailsheim, 2007), (for details see Becher *et al.*(Becher *et al.*, 2014)). Simulations for this study were carried out in two landscapes: i) a simple, theoretical landscape, consisting of just two patches, which clearly shows how the relative distance between the patches affects the visitation to each patch and the resultant impact of any pesticide treatment; and ii) A complex landscape, based on a map, to see if any of the effects from the simple landscape still occur in a realistic landscape.

4.3.2.1 Simple landscape

The simple landscape consisted of two patches, a 'treated' patch which represents a theoretical pesticide treatment and an untreated patch (from hereafter called the 'background' patch) representing pesticide free background forage. The treated patch, which remained at 1km from the colony, represents a crop treated with a pesticide; and the untreated patch which was placed at either 500m, 1km or 1.5km, represents the general background forage available in the landscape

These patches provided a seasonal nectar flow, based on Schmickl and Crailsheim (Schmickl and Crailsheim, 2007) and both patches are set to peak in their food availability (Maximum of 20l of 1.5 Mol L⁻¹ nectar and 1kg pollen, as in Becher *et al.* (Becher *et al.*, 2014)) at the same time in the year, this is intended to simplify the scenario and remove any effect from having the fields available at different times.

This scenario will show how the relative distance between background forage and a treated patch affects the impact a pesticide treatment may have on a honeybee colony, for example a mitigation effect from the background patch being closer to the colony and therefore receiving the majority of the foraging force, and also how the overall distance to food can affect the colony.

4.3.2.2 Complex landscape

The complex landscape is a theoretical landscape based on the real landscape surrounding Rothamsted Research (Hertfordshire, UK), from a satellite photograph taken in 2009. This consists of 115 patches of forage (fields), with distance to the

colony and size of patch based on the data from around Rothamsted Research (Figure 1). These patches have been separated into three field types (Red, Blue and Yellow) based on the crop type in the patch in the real landscape. The three field types

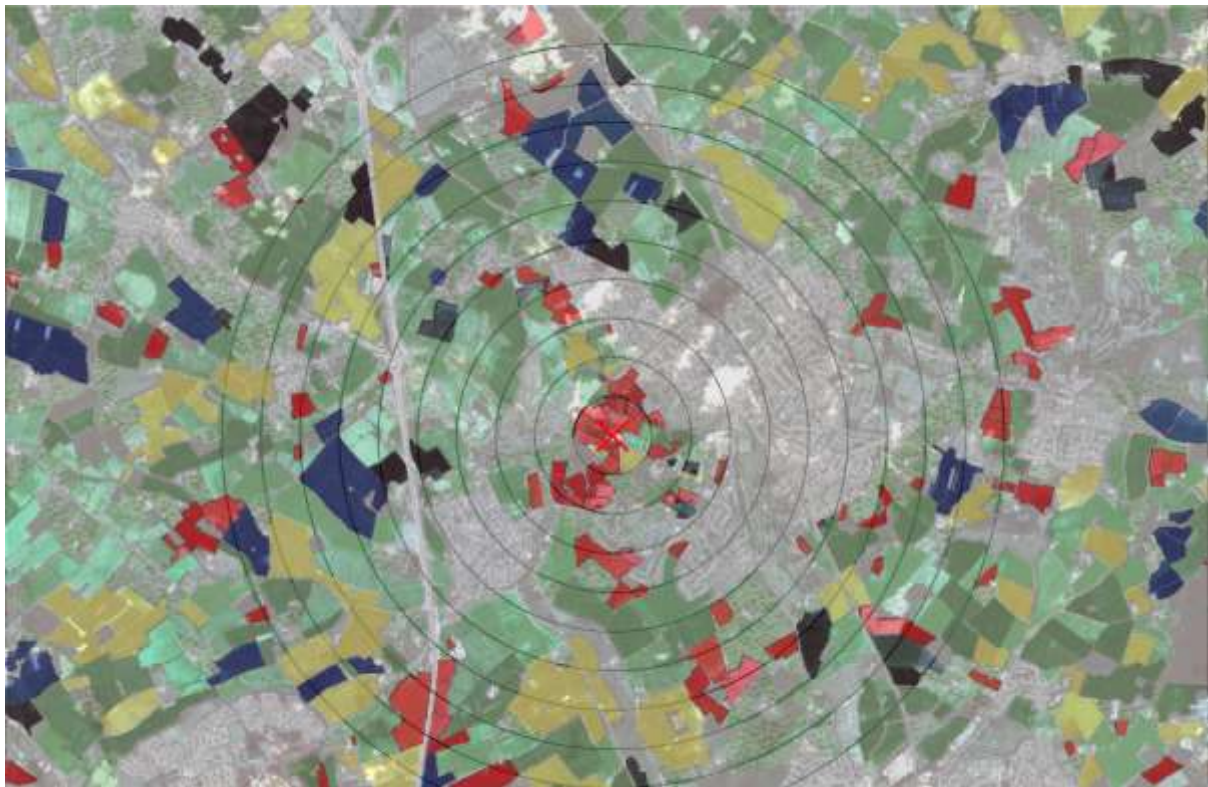


Figure 4-1

A- The placement of fields in the complex landscape, with the colony situated at the red cross. This is a satellite photograph of Rothamsted Research, taken in 2009. The distance from the colony and area for each crop were calculated and the crops were split into three types based on an estimate of the original crop type. Fields are colored according to their field type (red, blue yellow)

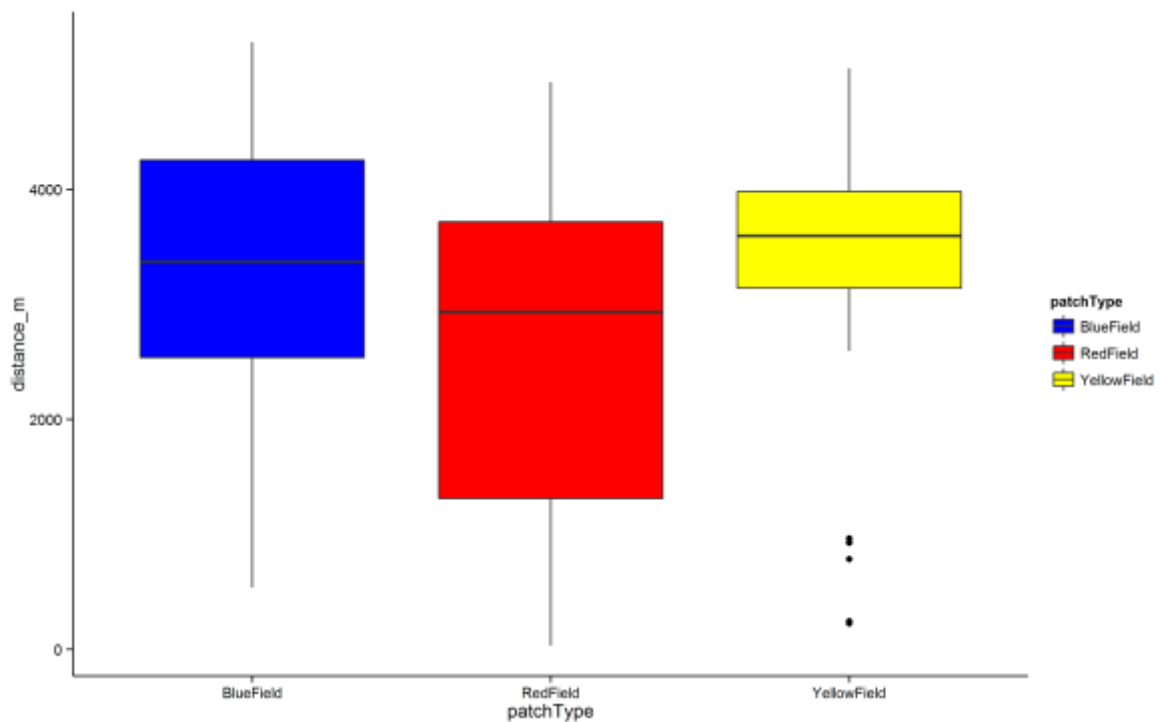


Figure 4-2

A box plot (Box showing 25th and 75th percentile, bar within box showing median, whiskers show the highest and lowest value within a distance of $1.5 \times$ interquartile range, of the box) showing the distance of the patches (in m) of the fields in the complex landscape from the colony arranged by field type.

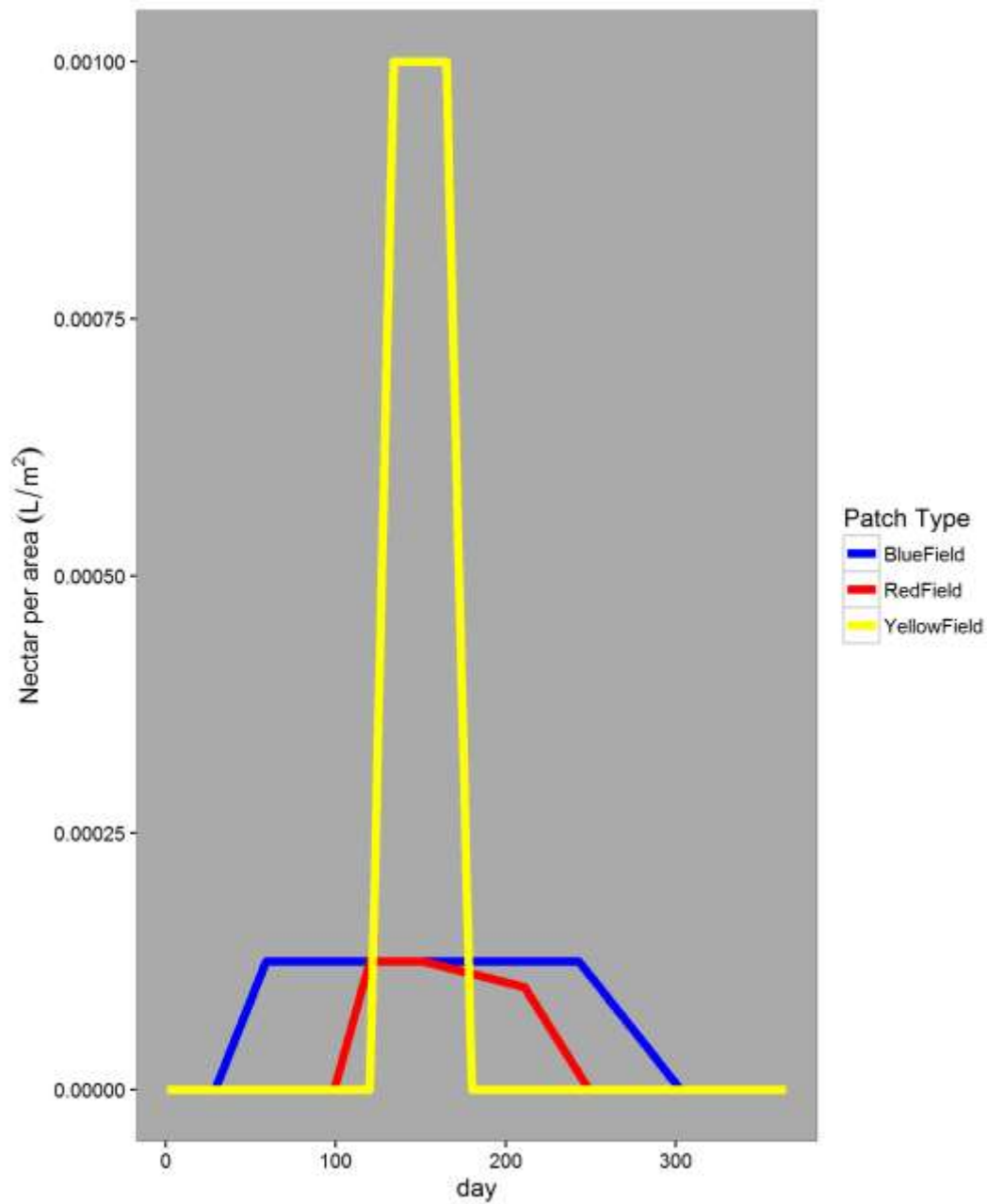


Figure 4-3

The average volume of nectar per m² of the three field types in the complex landscape: calculated as the total volume of nectar each of the patches provided divided by the total area of the patch. Patterns were chosen arbitrarily, but within the boundaries of real availability of real crops.

overlap in distance to the colony (Figure 4-2) and therefore provide a more realistic scenario for the foragers as opposed to the simple landscape. Each of the field types has a different flowering time (generally based on the crop type in the real landscape) and quantity of nectar (Figure 4-3) and pollen per unit area. In general, the red patches are in flower for the longest time, and are closer to the colony, offering high sugar concentration nectar (2 mol L^{-1}), but are the smallest patches and have the lowest volume of nectar. The yellow and blue patches offer nectar of the same sugar concentration (1.5 mol L^{-1}) and are further away and available for less time, but offer a higher volume of nectar per area and are larger. This landscape within the model was created from a satellite map of the area surrounding Rothamsted Research (this is a standard input file with the BEEHAVE2013 version of the model), crop locations were noted and their distance from the central point, where the theoretical colony is assumed to be, was calculated, as well as their x and y coordinates. The patches were split by their estimated crop type and assigned a 'field type'. The crop types were then assigned arbitrary nectar volumes per unit area and qualities to simulate a varied landscape. In our analysis we are not able to separate the impact of the distance of the patches to the colony and the nectar quality and quantity of the nectar, as they are confounded. As we are not altering this landscape in any simulation, just changing which of the patches are treated, this will not impact the analysis of the results, however, if the landscape were to change in organization, they would need to be separated. When simulating a pesticide exposure, one can choose which of the field

types is 'treated' and becomes associated with the pesticide effects described below. This scenario is designed to show how treating different crops in a complex landscape affects the impact of the pesticides in those crops and seeks to explore how the presence of attractive crops close to the colony in a complex landscape affect the colony, both when they are treated with a pesticide and when other, potentially less attractive crops are treated with a pesticide.

4.3.2 Pesticide Treatments

Two different impacts from a theoretical pesticide at the treated patch were investigated: i) a direct mortality effect, increasing the mortality of the foraging bees, either for 30 days in one of 3 months, or killing all foraging bees for 1 day each month; and ii) a sublethal effect, simulated as reduced foraging time.

4.3.2.1 Direct mortality

We ran simulations to compare two modes of direct mortality from a pesticide. In both, the route of exposure is assumed to be pesticide contaminated nectar or pollen collected from the food patch. BEEHAVE calculates background foraging mortality by taking into account factors such time spent foraging and distance flown and this is patch specific. To represent pesticide related mortality, the mortality associated with the treated food patch was altered, this is modelled as the percent chance of a single forager (or forager squadron) becoming lost or dying on the journey back from the food patch to the colony. Firstly, we simulated a long lasting exposure (Chronic), representing, for example, a systemic pesticide in the nectar or pollen of a crop from a seed coating. This is assumed to remain in the nectar and pollen for an extended

period but not in high enough levels to affect all foragers. Secondly, we simulate a single, highly toxic event (Acute), such as a foliar spray of an insecticide which is highly toxic, killing all foragers, but does not persist in the food patch. To provide a highly extreme example of repeated treatments as an example of the principle, the pesticide is applied monthly to the food patch.

4.3.2.1.1 Chronic exposure

For the 'chronic exposure' treatment, a 5% mortality per foraging trip was applied to the foragers visiting the treated patches for 30 days. This is the mortality that each forager experiences on each foraging trip, This value was chosen based on results from Rumke et al. (Rumke *et al.*, 2015)(Chapter 2) as a level of foraging mortality that causes the average colony to suffer noticeable reduction in population without failing. This treatment period began on either 1st April, 1st June or 1st August. This scenario represents a single treatment of a pesticide with low acute but high chronic toxicity and which is available to the foragers for an extended period.

4.3.2.1.2 Acute exposure

For the 'acute exposure' treatment, a 100% mortality per foraging trip was applied to the foragers visiting the treated patch for one day. This was repeated on the first day of each month. This scenario represents multiple treatments of one or a number of high toxicity pesticide which does not persist on the crop, for example, a non-systemic pesticide being sprayed during foraging (Johnson, 2015). This is an extreme scenario, unlikely to represent a specific treatment scenario in the real world, but used here to test a principle.

4.3.2.2 Sublethal effect

To simulate a general sublethal impact to the foragers, the amount of time available for the foragers to forage was reduced to 75%, 50% and 25% of the default value. This represents an arbitrary sublethal effect of a pesticide in the colony reducing the foraging activity of the bees.

4.3.3 Simulations

Each simulation was run for 2 years with the same forage and pesticide treatment (where applicable) occurring in both years, with data collected in both years. This allowed the colony to over-winter and we can then see whether any negative effects continue into the second year. Each scenario was run with 20 replicates each with a different seed given to the random number generator for each replicate (values 1-20 depending on the run). This provides stochasticity to each scenario to show a fuller range of responses (whilst also, if necessary, enabling the comparison of results with the same seed in different scenarios to remove this stochasticity).

4.3.3.1 Output

The maximum number of adult bees in each of the two years was used as a measure of colony success to investigate the impact of the pesticide impacts on the modelled colony. To give a quantitative measure to compare between scenarios, these values were used for each treatment and landscape (including the three variations of the two

landscapes), to calculate the mean maximum size of each colony in the treatment scenarios as a percentage of the mean maximum control colony size. To help understand the behaviour of the foragers in the different landscapes, the visitation rate of foragers to each of the patches was also recorded on each day.

4.4 Results

4.4.1 Direct mortality

4.4.1.1 Simple Landscape (Figure 4-4A)

In the simple landscape, the distance of the background patch to the colony had a very significant impact on the maximum colony population, both in the presence of a pesticide, but also when no pesticide was present due to the overall distance of available food to the colony. When the patches are both far from the colony, the chance of a forager becoming lost is higher than when there is a patch close to the colony, as the mortality increases with distance travelled. This resultant stress on the foragers leads to a reduction in food brought into the colony, as well as the requirement

Simple Landscape						
Exposure	Year 1			Year 2		
	500m	1km	1.5km	500m	1km	1.5km
Chronic - April	0%	-4%	-12%	-1%	-15%	-32%
Chronic - June	-3%	-29%	-36%	-6%	-54%	-75%
Chronic-August	0%	0%	0%	-3%	-15%	-31%
Acute	-7%	-18%	-30%	-12%	-47%	-63%
Complex Landscape						
Exposure	Year 1			Year 2		
	Blue	Red	Yellow	Blue	Red	Yellow
Chronic - April	-1%	-13%	-1%	-7%	-14%	+1%
Chronic - June	+1%	-28%	-2%	+2%	-37%	0%
Chronic-August	0%	0%	0%	0%	-2%	+1%
Acute	-6%	-3%	+1%	-4%	-9%	-4%

Table 4-1

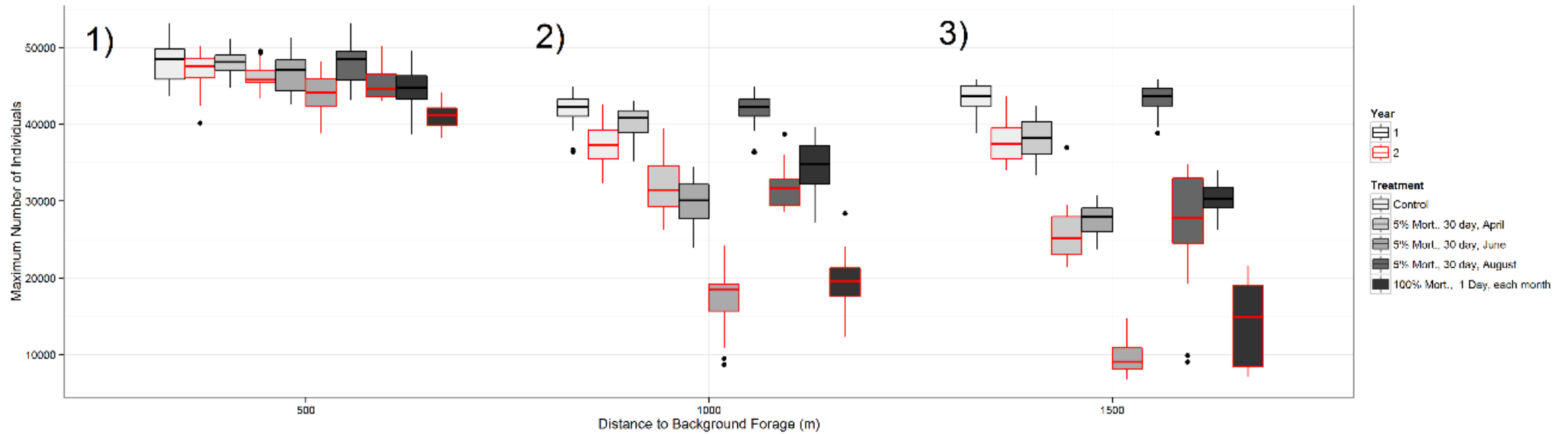
The percent change in maximum number of bees compared to the control, for each pesticide treatment in each of the direct mortality scenarios. Distances are from the colony to the background (non-treated patch) and colours represent the patch type treated in the complex landscape

	Simple Landscape			Complex Landscape		
Mortality	500m	1km	1.5km	Blue	Red	Yellow
Chronic - April	0	0	0	0	0	0
Chronic- June	0	3	15	0	2	0
Chronic-August	0	0	3	0	3	0
Acute	0	1	10	0	1	0

Table 4-2

The number of colonies that died, or had a population of < 4000 bees on the 31st December of the second year (the assumed threshold for over-winter survival) for each of the direct mortality scenarios. Distances are from the colony to the background (non-treated patch) and colours represent the patch type treated in the complex landscape

A) Simple Landscape



B) Complex Landscape

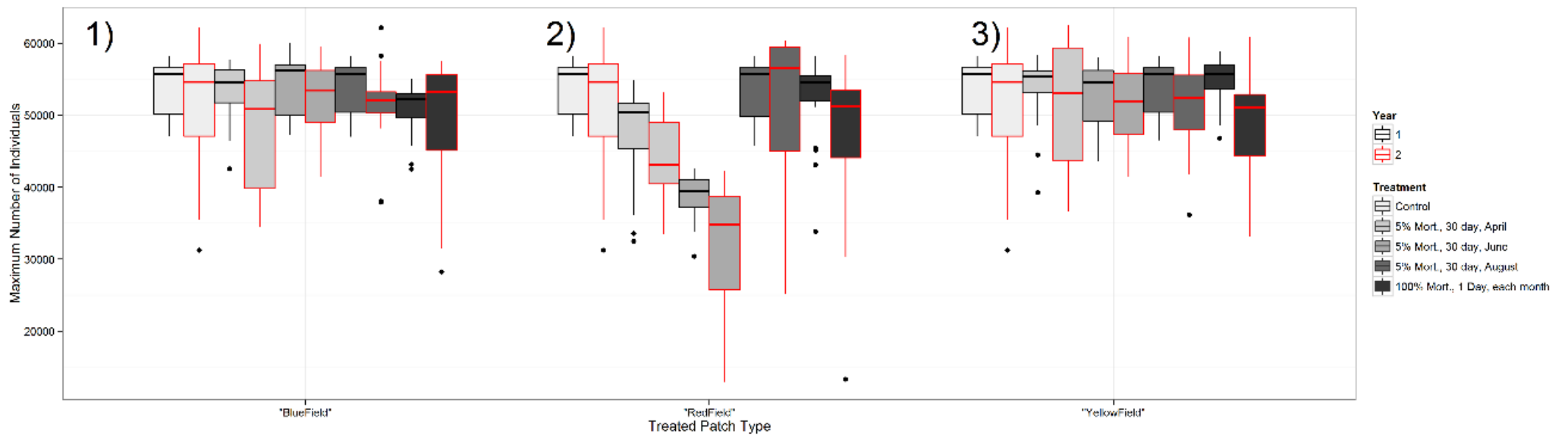


Figure 4-4

Direct mortality: chronic exposure (grey bars) and acute exposure (black bar). Box plots (Box showing 25th and 75th percentile, bar within box showing median, whiskers show the highest and lowest value within a distance of $1.5 \times$ interquartile range, of the box) showing maximum number of bees per colony in years one (black bordered) and two (red bordered) in the control and pesticide treatment scenarios (see key). A) for the simple landscape, B) for the complex landscape.

for younger bees to become foragers, reducing the number of in-hive bees available to perform tasks such as brood rearing.

When the background patch is closer to the colony than the treated patch (Fig 4-4A.1), colonies in all treatment scenarios saw a small decrease in maximum population size from year one to year two. In addition, none of the pesticide treatments led to a large drop in maximum population size in either year, with a percentage change in maximum population size (compared to control) of between 0% and -7% in year 1 and between -1% and -12% in year 2 (Table 4-1). No colonies were lost for any of the chronic exposure treatments, (Table 4-2).

When both patches are the same distance (1km) from the colony (Fig 4-4A.2), there is a reduction in maximum population size from year one to year two, regardless of the treatment. A chronic 30 day exposure causing of 5% foraging mortality per trip in April led to a small reduction in the maximum population size in year one (-4% change from control), and a larger reduction in year two (-15% from control). The same 5 % mortality per trip for 30 days in June, had a larger impact on the maximum population size (Year 1: -29% from control, Year 2: - 54% from control) and three colonies were lost (Table X). When this chronic 30-day period was in August, however, there was a reduction in maximum population size for the second year (-15.1% from control), but not the first. The maximum size of a colony is reached during August and so this impact may not have time to show. An acute 100% foraging mortality on the first day of each month led to a reduction in the maximum population size in both years (Year 1: -15%, Year 2: -47%) and one colony being lost (Table 4-2).

When the background forage patch is further from the colony (at 1.5km) than the treated patch(Figure 4-4A.3), regardless of treatment, there is a reduction in maximum population size from year one to year two. In all cases this reduction was larger than in the previous scenarios. A chronic 30 day exposure period of 5% foraging mortality per trip in April reduced the maximum number of bees in the colony in both years (Year1: -12%, Year 2: -32%). A chronic 30 day period of 5% foraging mortality in June led to a reduced maximum population size in both the first and second years, compared to the control (Year 1: -36%, Year 2: -75%) and 15 colonies lost (Table 4-2). A 30 day period of 5% foraging mortality in August, however did not show a reduction in maximum population size until the second year, compared to the control (Year 1: 0%, Year 2: -31%), and resulted in 3 lost colonies (Table 4-2). An acute 100% foraging mortality on the first day of each month led to a substantial reduction in maximum population size in both years (Year 1:-30%, Year 2: -63%) and 10 colonies being lost.

4.4.1.2 Complex landscape (Figure 4-4B)

In the complex landscape based on a real-world landscape, if either the blue or yellow fields were treated, with either a chronic or acute exposure (Figure 4-4B.1&3), there was little impact from any treatment tested (largest change of -6% - blue field treated, acute exposure). There also did not seem to be any reduction in maximum number of bees/colony in any scenario from year one to year two, and no colonies were lost (Table 3-3). When the red fields were considered to be the treated patches (Figure 4-

4B.2), a chronic 30-day 5% foraging mortality per trip in August or an acute 1-day 100% mortality on the first day of each month had little impact on the maximum population size in either year, however an acute mortality led to one colony becoming lost and a chronic exposure in August led to 3 colonies becoming lost. A 30-day 5% mortality per trip in April or June however, led to a reduction in maximum population size, compared to the control in both years, with a June treatment having the most severe impact, including two colonies becoming lost (Table 4-2)(April – Year 1:87% of control, Year 2: 86% of control; June – Year 1:72% of control, Year 2: 63% of control;)

.

4.4.2 Sublethal effects – Reduced Foraging (Table 4-3)

4.4.2.1 Simple Landscape (Figure 4-5A)

A 30-day reduction in the daily available foraging time had little effect when applied in April or August, with the largest average impact found being a reduction to 92.6% of the control (August treatment, untreated patch at 1.5km, 2nd year). In all cases the maximum colony population was lower in the second year than in the first year. When the sublethal effect is applied in June, however, there was a noticeable impact from the reduction in available foraging time, with the largest effect being a reduction to 57% of the control (25% of time available to forage, background patch at 1.5km, 2nd year). When the foraging time is reduced to 50% or 75% there is little .

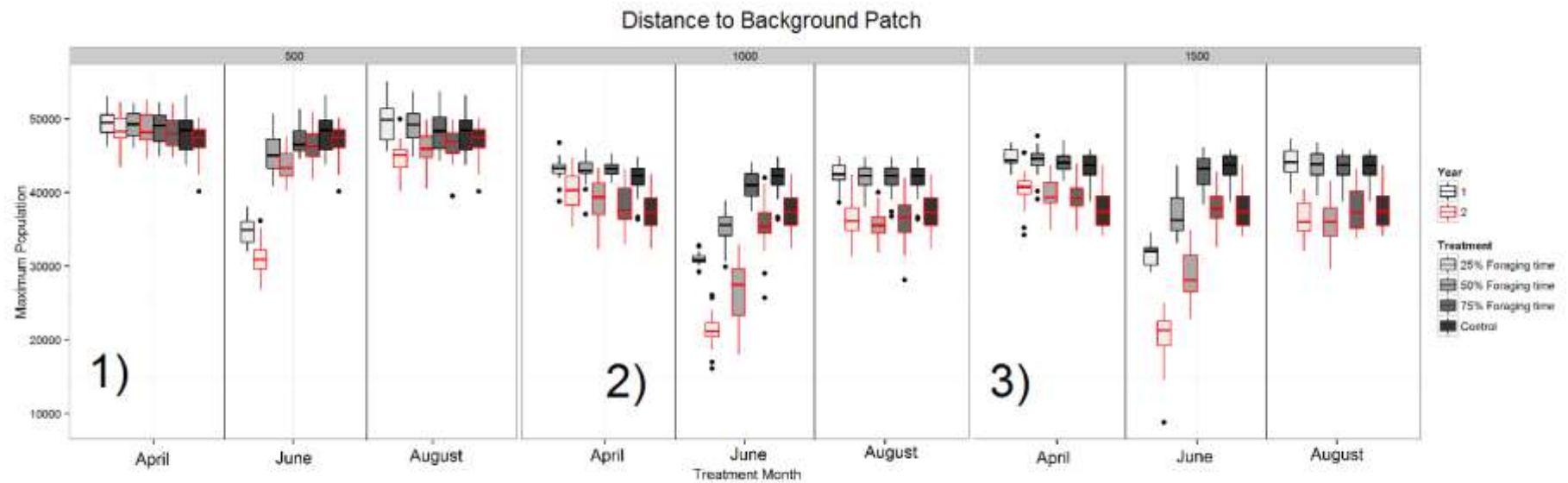
Simple Landscape		Year 1			Year 2		
Treatment Month	% available forage time	500m	1km	1.5km	500m	1km	1.5km
April	75%	-2%	+1%	+3%	+4%	+2%	+1%
April	50%	-2%	+3%	+4%	+5%	+2%	0%
April	25%	0%	+4%	+2%	+4%	+5%	+4%
June	75%	-3%	-1%	+1%	+2%	-1%	-2%
June	50%	-5%	-13%	-5%	-5%	-22%	-20%
June	25%	-28%	-27%	-25%	-28%	-39%	-43%
August	75%	+1%	0%	0%	-1%	-1%	-1%
August	50%	+1%	0%	+2%	-1%	-6%	-5%
August	25%	+4%	0%	+3%	0%	0%	-7%

Complex Landscape		Year 1			Year 2		
Treatment Month	% available forage time	Blue	Red	Yellow	Blue	Red	Yellow
April	75%	+2%	+2%	+2%	-1%	-1%	-1%
April	50%	+3%	+3%	+3%	0%	0%	0%
April	25%	+1%	+1%	+1%	0%	0%	0%
June	75%	-3%	-3%	-3%	-2%	-2%	-2%
June	50%	-7%	-7%	-7%	-3%	-3%	-3%
June	25%	-11%	-11%	-11%	-15%	-15%	-15%
August	75%	0%	0%	0%	-3%	-3%	-3%
August	50%	0%	0%	0%	-11%	-11%	-11%
August	25%	0%	0%	0%	-4%	-4%	-4%

Table 3-3

The percent change in maximum number of bees in the colony when a reduction in the available time to forage was applied in for 30 days, when compared to the control (no reduction applied). Distances are from the colony to the background (non-treated patch) and colours represent the patch type treated in the complex landscape

A) Simple Landscape



B) Complex Landscape

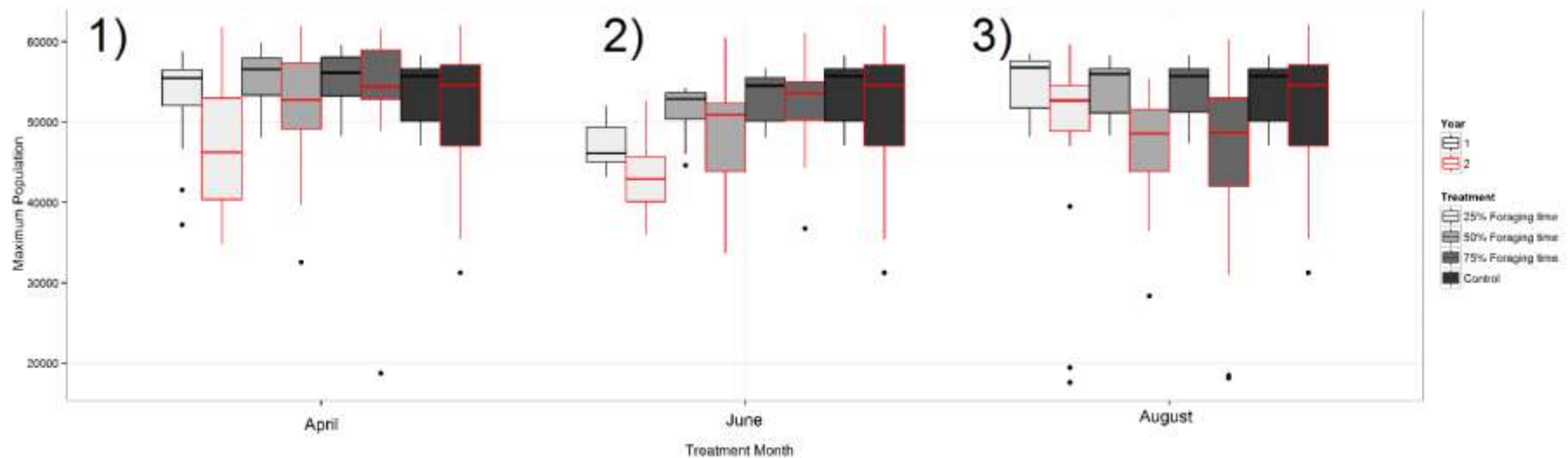


Figure 5-5

Sublethal effects. Box plots(Box showing 25th and 75th percentile, bar within box showing median, whiskers show the highest and lowest value within a distance of $1.5 \times$ interquartile range, of the box) showing Maximum number of bees/colony in years one (black bordered) and two (red bordered), with sublethal exposure modelled as varying levels of reduced foraging time per day (see key). A) gives values for the simple landscape, B) gives values for the complex landscape. The x axis of each panel shows the month in which the impact was applied. In A, the different panels show the data for the three landscape variations (distance to background patch). For B, this is not necessary, as the landscape did not change and the sublethal impact

difference in the percent reduction in maximum population compared to the control in either year. When the daily available foraging time was reduced to 25%, there was a severe reduction in maximum number of bees in both years (change from control of between -25% and -43%). When the background forage was equidistant for further from the colony than the treated patch, there was a much more noticeable impact from a reduction in available foraging time (other than a change by -25% compared to control). The impact of the landscape on the change in maximum number of bees compared to the control is more noticeable in the second year than in the first.

4.4.2.2 Complex Landscape (Figure5- 5B)

The effect of the reduction of the foraging time available did not depend on the type (colour) of patches that the foragers were visiting. As such there was no effect of the type of patch being treated. When the amount of time available to forage was reduced in April, there was little impact on the maximum number of bees in the colony in either year (largest reduction -1%), regardless of the magnitude of the reduction. When applied in June, however, there again was little impact, except for the 25% foraging time scenario, in which reductions of the maximum number of bees in the colony of -11 % and -15% of control were seen in years 1 and 2 respectively. When applied in August, there was little impact, except for a 50% foraging time scenario, in which the maximum number of bees in year 2 was -18% of the control.

4.4.3 Visitation of foraging bees to forage patches

4.4.3.1 Simple Landscape

In the simple landscape, when the background patch is closer to the colony than the treated patch (Fig. 4-6A-C), the majority of foraging visits are to the background patch, although there is still some visitation at the treated patch. There is little effect of the timing of a chronic exposure.

When both patches were equidistant from the colony (Fig. 4-6D-F), there were similar levels of visitation at both patches. When the background patch is further from the colony than the treated patch (Fig. 4-6G-I), the majority of foraging visits were to the treated patch. There were still visits to the background patch, however there appear to be fewer visits to the background patch in this scenario than there were visits to the treated patch in the scenario in which the treated patch is further from the colony. Although there is the same distance between the patches in these two scenarios, when the treated patch is at 1.5km from the colony, the overall distance to any food is increased, leading to more stress on the foragers together with the pesticide related mortality, as well as longer foraging trips, reducing visitation.

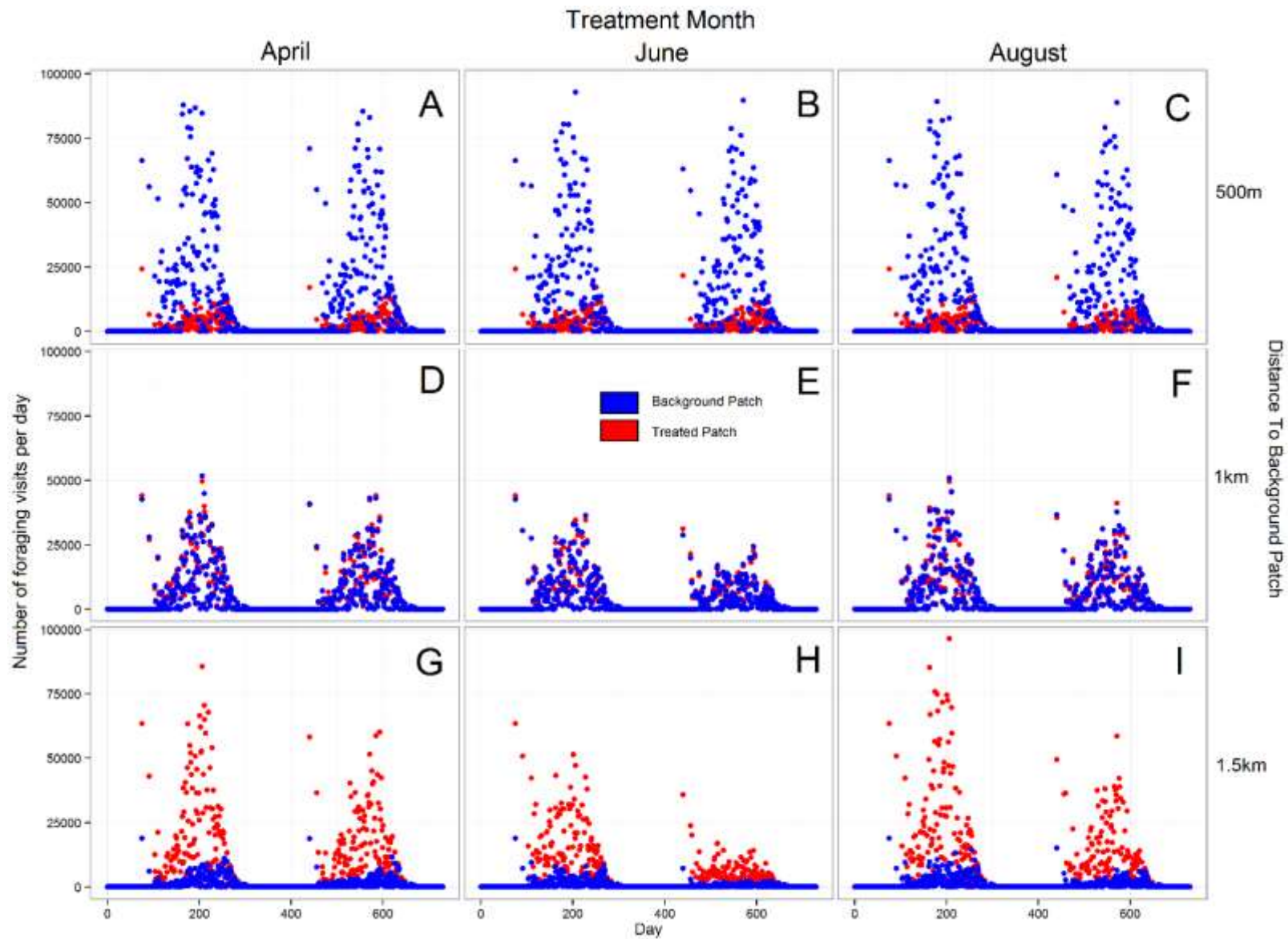


Figure 4-6
Number of foraging visits per day to the treated (red) and background (blue) patches in the simple landscape. Columns show data for the three treatment months and rows show data for the three landscape configurations.

4.4.3.2 Complex Landscape (Figure 4-7)

In this landscape, the blue fields are in flower (available for foraging) for the longest period (Figure 4-3), the red fields for a shorter period and the yellow fields for the shortest time (Figure 4-3). The red fields are much closer to the colony, on average than the other two colours, with the blue patches being, on average, slightly closer than the yellow patches (Figures 4-1 & 4-2). The distance to each patch, as well as that patches flowering time and nectar quality and availability are fixed, and so these effects cannot be separated. Therefore when they are in flower, the red patches receive the majority of the foraging visits, otherwise, if they are available, the blue patches receive the majority of the visitation. The yellow and red patches are in flower at the same time, and although the yellow patches, on average, provide a much larger quantity of nectar per unit area (Figure 4-3), the proximity of the red patches mitigate any increased foraging attention the yellow patches would receive. There is little impact on visitation of the timing of the pesticide application, or the field type being treated, except that there is a reduction in visitation in the scenario in which the red patches are treated in June.

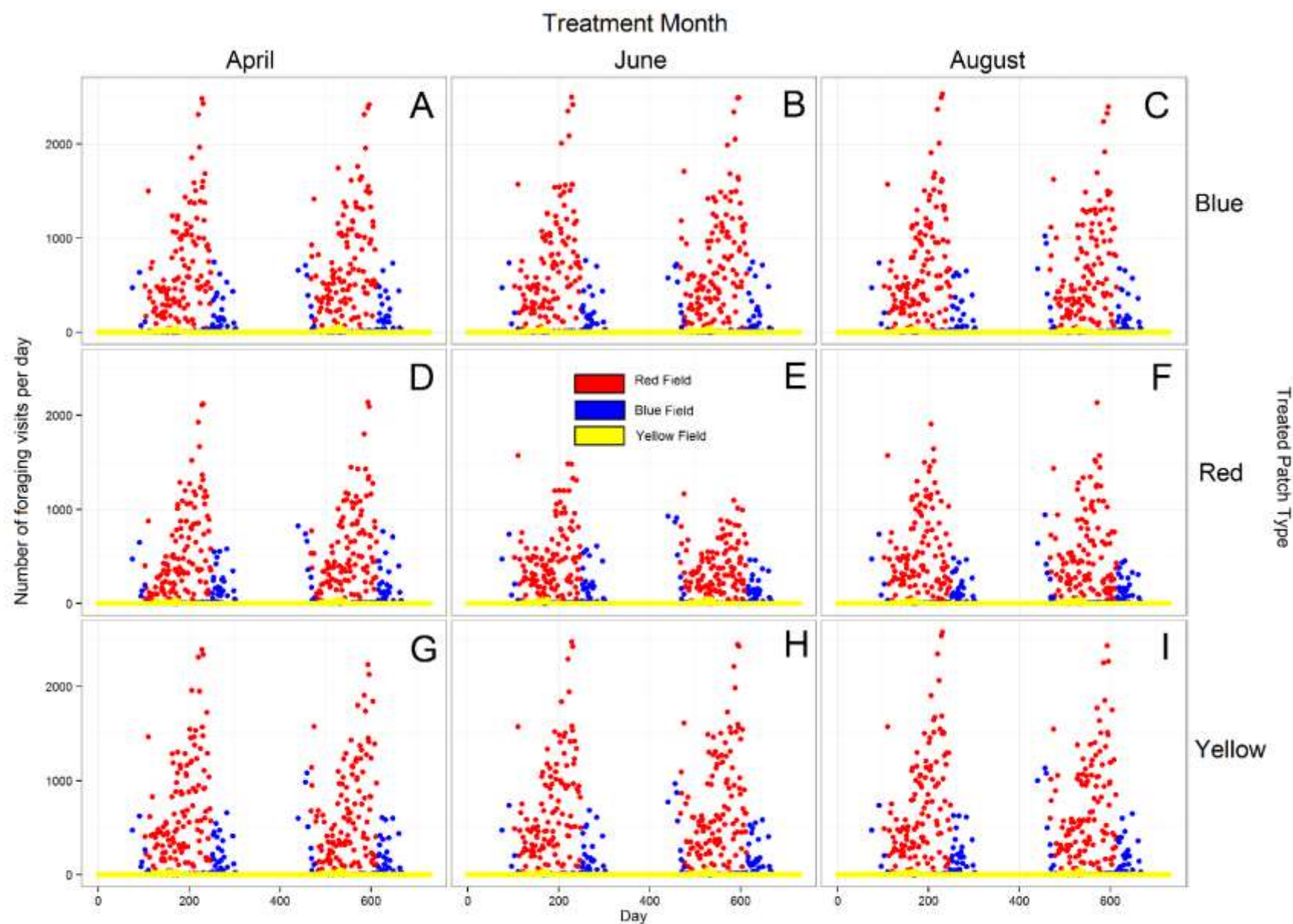


Figure 4-7

Number of foraging visits per day to the three field types (red, blue and yellow) in the complex landscape.

Columns show data for the three treatment months and rows show data in scenarios in which each of the field types was the treated field type.

4.5 Discussion

We investigated the impact to the honeybee colony of increased mortality of actively foraging bees resulting from two theoretical pesticide exposure scenarios (chronic and acute), as well as a sublethal effect on foraging behaviour in two different landscapes each of which varied in terms of distance to forage as well as timing of forage availability. Our results suggest that both a chronic 5% foraging mortality per trip at the treated patch for 30 days and a single day of 100% foraging mortality for one day each month at the treated patch can significantly reduce the maximum number of bees a colony produces in a year. The timing of the 30-day period of 5% foraging mortality per trip (as shown also in Chapter 2 (Rumkee *et al.*, 2015)) has a strong effect on the resultant impact on the colony, as the colony size changes throughout the year and the amount of foraging activity occurring on any particular day changes depending on the weather. The landscape also has a major effect on the impact of the foraging mortality on the colony, with both the complexity of the landscape and overall distance to forage showing noticeable effects on resultant colony performance, even without any additional mortality from the pesticide treatment.

In a simple landscape of two patches, whichever of the patches is closest to the colony receives the majority of the forager visitation (Fig 4-6). This visitation is governed in the model by the chance of a forager to initially discover the patch, which will control the number of naïve foragers visiting the patch, as well as the energetic efficiency of the patch (a function of the distance to the patch from the colony, the handling time of the patch and the quality of the nectar at the patch). As both patches in the model have

the same nectar quality, the distance to the patches is the most important factor. In a complex landscape, in which there are a large number of patches, with a flowering time dependent on the crop type, a similar outcome is found. In the complex landscape used for these simulations, the red fields are on average closer to the colony than the other two crop types (Figure 4-2). While these fields are in flower, they still receive the majority of the foraging visits, despite not providing the highest volume of nectar per area, as in this landscape, the distance to the colony is the most important factor.

In the simple landscape, the magnitude of the impact of the pesticide treatment on the colony increased as the background (untreated) forage was moved away from the colony. When the background forage was closer to the colony than the treated forage, as the majority of the foraging visitation was to the non-treated background patch, there was little impact on the colony from any of the pesticide treatments. As the background patch was moved away from the colony and the proportional visitation of the foragers to the treated patch increased, the impact on the colony from the pesticide treatment increased. Both when the background patch was at 1km or 1.5km, a 30-day period of 5% foraging mortality per trip in April or August had a roughly equivalent impact on the colony in both years (Table 1). In the complex landscape, the same pattern occurs when the red fields are treated. During April the colony has yet to begin increasing in size, and any small losses are compensated for as the colony increases in size in the summer (Figure 4-8). During August, the colony has reached the maximum size for the year and begins reducing in size for the winter period (Figure 4-8), and so, as long as the population does not fall to the point at which the colony

cannot survive the winter, there is little impact on the colonies ability to grow again the following year.

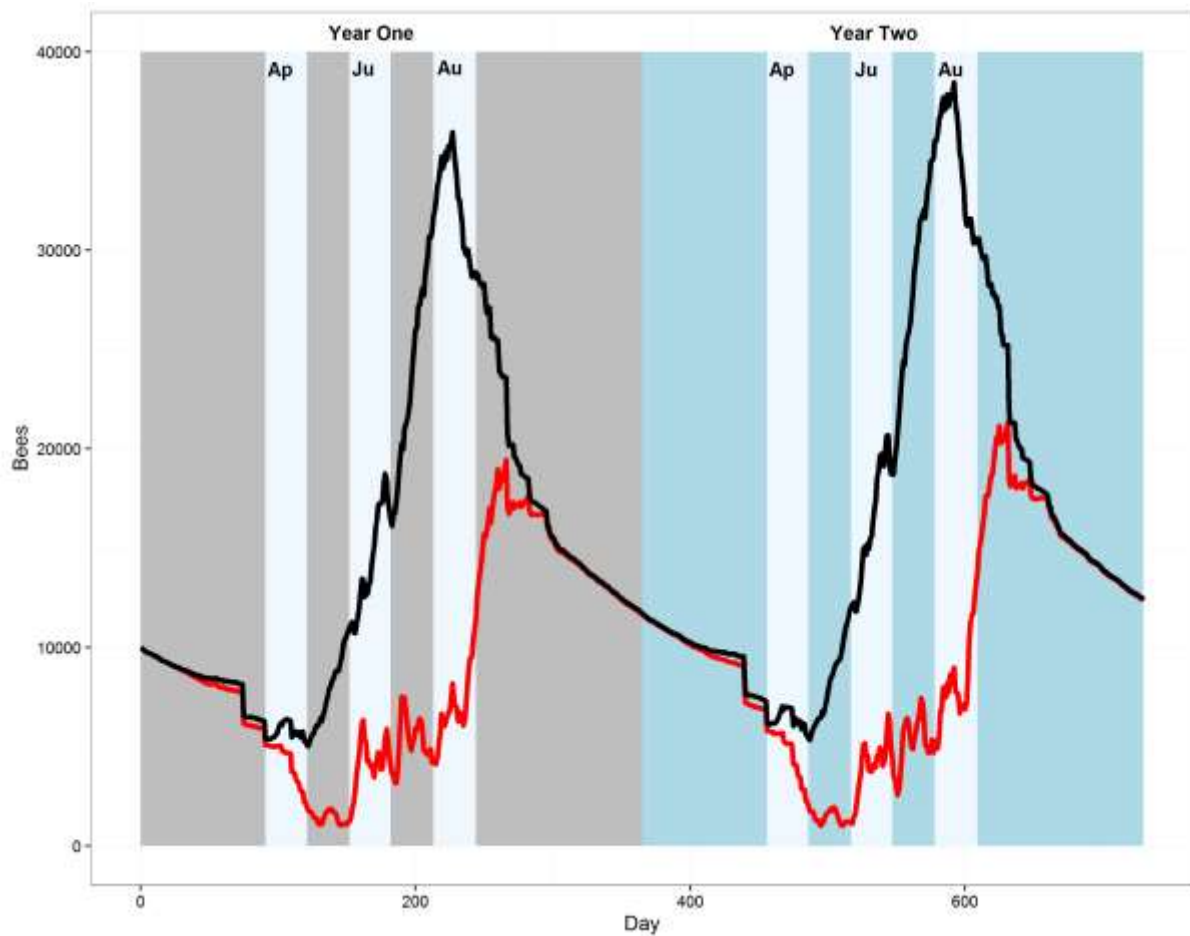


Figure 4-8

Total adult bees (black) and foragers (red) in the simple landscape when both patches are equidistant and there is no pesticide treatment, data taken from a single simulation of a single colony. The three treatment periods (Ap – April, Ju – June, Au – August) are shown.

During June, however, there are a lot of foraging trips taking place and both forager and in-hive bee numbers are increasing (Figure 4-8). Any disturbance to the forager population during this time has a noticeable impact on the forager population and total adult numbers (Figures 4-9 & 14-0)(Also shown in Chapter 2 (Rumkee *et al.*, 2015)). As the forager population decreases, younger, in-hive bees are recruited to become foragers, to maintain a foraging force able to collect enough food, this adds stress within the colony as there are then fewer in-hive bees able to perform duties such as brood feeding. When a 100% mortality was applied for a single day on the first day of each year, representing an extreme simplification of a non-systemic pesticide being applied via a spray repeatedly to a crop during foraging, there is a significant impact on the colony. The resultant impact is likely a result of a similar mechanism as with the chronic exposure, forager loss leading to younger bees becoming foragers and leaving fewer individuals to perform in-hive tasks.

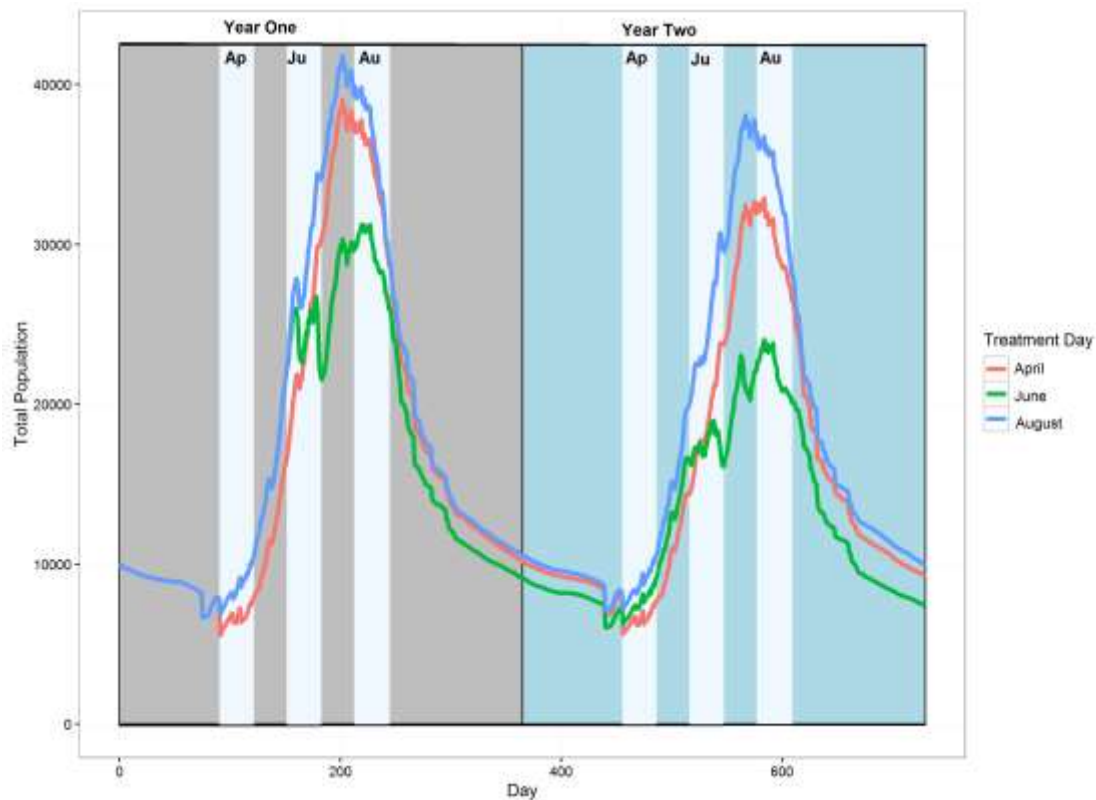


Figure 4-9

Total bees in colony in the simple landscape under the three 30 day 5% foraging mortality treatments, data for each line taken from a single simulation of a single colony. Each line represents a simulation with the treatment applied in a different month. The two patches were equidistant.

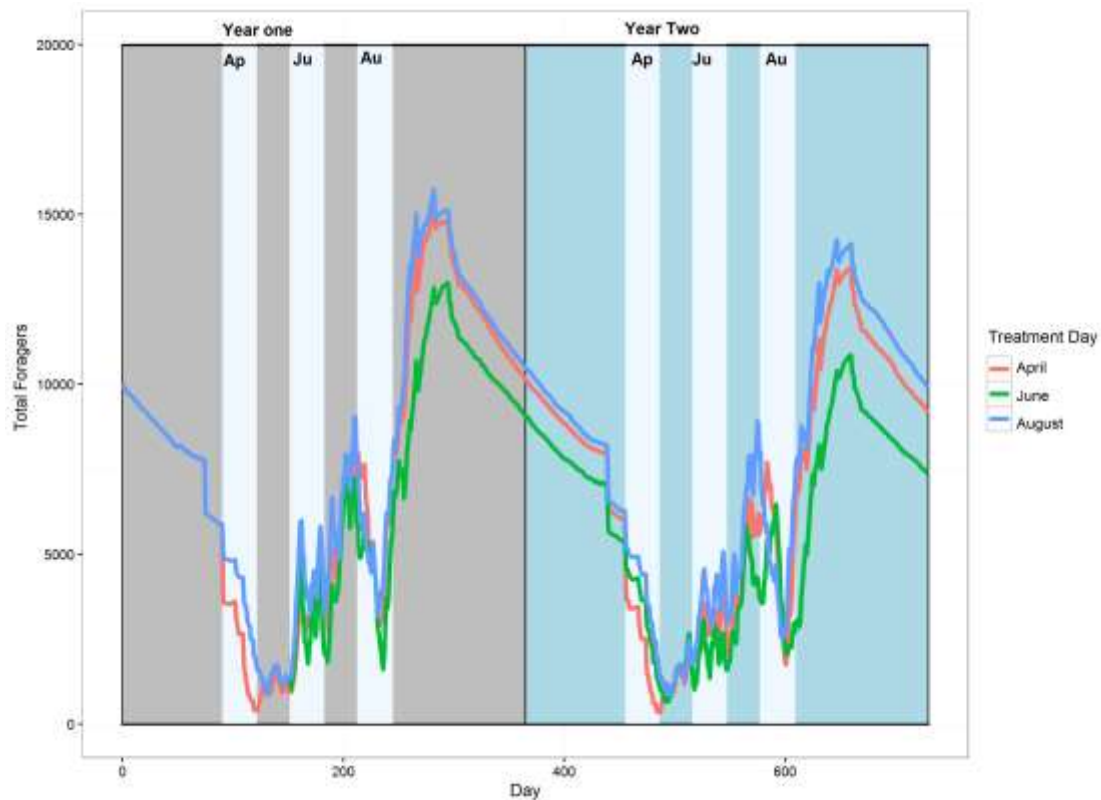


Figure 4-10

Total number foragers in a colony in the simple landscape under the three 30 day 5% foraging mortality treatments. Data for each line is taken from a single simulation of a single colony. Each line corresponds to a simulation in which the pesticide treatment was applied in a different month. The two patches were equidistant.

In general, in the simple landscape, the overall distance to the forage has a noticeable impact on the maximum colony size in both years (Figure 4-4). When the background patch is 500m from the colony, the colony is able to reach a higher maximum population size than when the closest patch is 1km from the colony. Similarly, in the complex landscape, maximum population sizes are higher than in the simple landscape in general, as there is more food available, closer to the colony.

Sublethal effects on foraging were much more noticeable in the simple landscape than in the complex landscape. In the simple landscape, there was little impact when the foraging reduction was applied in April or August. Reducing the time available to forage in June, however, has a much greater impact on the colony. During April there is little foraging occurring and during August, although there is still a lot of foraging, the colony has already collected sufficient food stores that it can withstand the reduced foraging period. During June, however, the colony is collecting a lot of food to support the growth of the colony, reducing the time available for this collection has a significant impact on the colony development which worsens as the background patch is moved away from the colony, increasing the time it takes to complete a single average foraging round. In the complex landscape, there was a similar pattern with little impact from an effect applied in April or August, but a noticeable impact (up to a change of -15% of the control) when applied in June. The exception to this was when a 50% reduction in the foraging was applied in August, which led to the maximum number of bees in the colony being reduced by 11% from the control in year two. Currently, the protection goals set by EFSA ((Efsa), 2013b), dictate that “*the magnitude of effects on colonies should not exceed 7% reduction in colony size*”. These results show that, for

the direct mortality, in a beneficial landscape (background patch closer to treated patch in a simple landscape or blue or yellow treated patches in the complex landscape) a chronic exposure (as applied in the simulations) does not exceed this. If the treated patches are closer to the colony, or if there are many acute exposure events, then this protection goal is not met. For a sublethal impact, the results show that reduced foraging in June, and possibly in August (Table 4-2) can lead to the protection goals not being reached, but otherwise, it is met.

The results presented here show that both a single month of a low level foraging mortality and multiple short instances of a very high foraging mortality can both severely disrupt the development of a honeybee colony. This highlights the importance of ensuring both that levels of systemic pesticides in the landscape are low enough to not lead to this level of mortality and that sprayed pesticides are not applied to crops in flower, or if it is unavoidable, that this occurs outside of the period when foragers will be active. Field studies presented by Rolke et al. (Rolke *et al.*, 2016), Rundlöf et al. (Rundlöf *et al.*, 2015) and Pilling et al. (Pilling *et al.*, 2013) show that at ‘field realistic’ levels, honeybee colonies appear resilient to commonly applied systemic pesticides implying that this is the case. Similar results were found by Thorbek et al. (Thorbek *et al.*, 2016) using the BEEHAVE model: they multiply the default foraging mortality by 2, 3, 5, and 10, reinforcing the importance of the landscape when assessing the impact of a pesticide on the honeybee colony. A beneficial landscape, providing plenty of food without any negative stress (i.e. from a pesticide) associated with it, can mitigate

pesticide impacts on the foragers, depending on the attractiveness of those untreated crops in the landscape. Whereas a sparse landscape can exacerbate the impact of pesticide stress to the foragers and reduce the colony size. It is clear that solely considering a pesticide is not enough to understand the risk a landscape poses to a honeybee colony, the presence or absence of a particular pesticide in one of the potential forage patches with the respective effect that will have on foraging bees is not a reliable predictor of how a colony in that landscape will fare.

Chapter 5 - Comparison of the Varroa module of the BEEHAVE model with empirical data from two different climates to improve the simulation of varroa dynamics

5.1 Abstract

The varroa mite (*Varroa destructor*) is an ectoparasitic mite of the honeybee and is thought to be a leading cause of honeybee colony losses. Determining how the mite impacts the colony dynamics and predicting when (potentially harmful) varroicide treatment is necessary are not simple tasks. As a result, the use of modelling, especially models incorporating many in-hive processes and stressors is a valuable tool. In this chapter, I present work towards the validation of the BEEHAVE model in the context of the population dynamics and impact of the varroa mite. A review of the BEEHAVE model by EFSA found that the model underestimates the impact of varroa, I further explore this while including a direct mortality on the bees from the mites, altering the mite reproduction model and applying a global density dependence on the mites. To test the model, I use two datasets, one from a study in the UK and one from a study in the USA. I find that the model captures some important patterns in the empirical data, however the BEEHAVE model does underestimate the impact of the varroa mite, and further calibration is needed and discussed. Importantly, I show that

the model is suitable for use in multiple climates, further highlighting the potential of the BEEHAVE model in honeybee research,

5.2 Introduction

The European honeybee (*Apis mellifera*) is an important pollinator providing a substantial proportion of the insect crop pollination (Morse and Calderone, 2000; Breeze *et al.*, 2011) an ecosystem service providing great agricultural (Klein *et al.*, 2007) and economic value (Gallai *et al.*, 2009). It is therefore of concern that there have been reports of declining colony health and increased over-winter losses of colonies (Aizen and Harder, 2009; Potts *et al.*, 2010; Burkle *et al.*, 2013), as this could potentially impact this ecosystem service. The managed honeybee is affected by a large number of stressors, some natural and some anthropogenic in nature (vanEngelsdorp and Meixner, 2010). The anthropogenic stressors include: i) the reduction of forage available in the landscape, possibly as a result of an increase in crop monocultures and reduction of natural flower resource; and ii) the use of insecticidal chemicals on crops (Johnson *et al.*, 2010; Johnson, 2015), which the colony feeds from which may either kill the foraging bees or those in the colony which feed on the contaminated nectar and pollen entering the colony (Krupke *et al.*, 2012), or can potentially cause sub-lethal effects (Decourtye *et al.*, 2005; Desneux, Decourtye and Delpuech, 2007; Schneider *et al.*, 2012; Mengoni Goñalons and Farina, 2015), impacting the behaviour or development of individuals.

In addition to those stressors explicitly resulting from human behaviour, there are also biotic stressors, such as parasites, disease and predation that are 'natural' but can be

promoted by human behaviour implicitly, due to the managed nature of the bees. The major biotic stressor, which we focus on in this study, is the ectoparasitic mite, *Varroa destructor* (Anderson and Trueman, 2000), which has been found to be highly correlated with colony losses (Genersch *et al.*, 2010; Kielmanowicz *et al.*, 2015), and damages the honeybee not solely through physical damage and feeding (Annoscia, Del Piccolo and Nazzi, 2012), but also as a vector of a number of diseases (Kevan *et al.*, 2006).

Varroa destructor is a haemophagous ectoparasitic mite that is a major threat to honeybee colonies (Rosenkranz, Aumeier and Ziegelmann, 2010). The mite has two distinct phases in its lifespan, the reproductive phase and the phoretic phase, each of which impact on a different life stage of the honeybee. Reproduction takes place within the cells of developing honeybee brood, which are invaded by female mites who lay an unfertilised egg. Due to the haplo-diploid gender regulation of the mite, this egg hatches into a male to fertilise subsequent eggs, producing females. While in the cell, these mites feed on the developing bee pupa. Once the developing pupa emerges as an adult bee, the female mites are released from the cell. When in the phoretic phase, the adult female mites attach themselves to an adult bee, feeding from the haemolymph of the bee. This attachment is preferential towards the nurse bees (Kraus, 1993), through chemical sensing. The preference of the mites to attach to the nurse bees provides more opportunity to drop into the cell of a developing larva.

The Varroa mite has the potential to seriously harm both the individual bee and the colony. This can occur through several means. Firstly, the mite acts as a vector for a

number of viral diseases (Shen *et al.*, 2005; Boecking and Genersch, 2008), with the presence of mites in the developing cells significantly affecting the viral load of the pupa (Khongphinitbunjong *et al.*, 2015). These viruses themselves can cause serious impacts (Highfield *et al.*, 2009) and even death of the individual bee. The presence of a varroa mite also impairs the immunity of the bee to viruses (Yang and Cox-Foster, 2005). Secondly, the mites attach themselves to individuals and feed on the haemolymph. This affects the growth of the pupa (Annoscia, Del Piccolo and Nazzi, 2012) and leads to smaller bees emerging. If a larval cell is invaded by multiple mites, the developing pupa is even smaller (Annoscia, Del Piccolo and Nazzi, 2012) and there is a negative correlation between the number of mites invading the cell and the eventual lifespan of the individual bee, implying that it is not just the presence of a mite, but the level of infestation that matters. The presence of varroa mites is also associated with a reduction in foraging and pollen gathering (Lach, Kratz and Baer, 2015). This reduction in foraging ability may further stress the colony, exacerbating other impacts of mite infestation.

Carrying out detailed empirical studies to investigate both the dynamics of the infested colony as well as the mite population dynamics, is difficult, especially if information regarding the potential synergistic impact of other stressors is also of interest or the aim is to carry the investigation out over multiple years. Due to these difficulties, the use of models for prediction or understanding is a valuable research tool. As the varroa mite is one of the most impactful stressors affecting the honeybee (Boecking and Genersch, 2008; Genersch *et al.*, 2010), which also acts alongside many other stressors, it is important that we have a model that is validated against data and able

to reliably capture the behaviour of a colony under known levels of stress, thus enabling us to answer important questions regarding varroa infestation, treatment, and mitigation..

There have been several models created explicitly to explore the bee-mite-virus complex in honeybees. Sumpter and Martin (Sumpter and Martin, 2004) and Ratti et al. (Ratti, Kevan and Eberl, 2015) present mathematical models, with seasonal effects implemented via different parameter values depending on the season. Ratti et al. (Ratti, Kevan and Eberl, 2015) focus on Acute Bee Paralysis Virus (ABPV) and find that when mites and the virus are present, the colony is likely to fail, but when there is no ABPV, stability can occur in the presence of a varroacide of sufficient efficacy. Sumpter and Martin (Sumpter and Martin, 2004) explore both ABPV and Deformed Wing Virus (DWV) and find that autumn is the time at which the colony is most at risk to the virus, that the viruses require different mite treatment strategies to control, and both can lead to colony loss. Kang et al. (Kang *et al.*, 2016) present a model combining ideas from the models in Sumpter and Martin (Sumpter and Martin, 2004) and Ratti et al. (Ratti, Kevan and Eberl, 2015) and find that the initial bee population is very important in the resultant impact of an infestation and also, as shown in the model presented by Eberl et al. (Eberl, Frederick and Kevan, 2010), that the adult bee to bee brood ratio of the colony is important in maintaining the colony through infestation. DeGrandi-Hoffman and Curry (DeGrandi-Hoffman and Curry, 2004) present a model of bee and mite dynamics, but with no explicit virus. This model is used in DeGrandi-

Hoffman et al.(DeGrandi-Hoffman *et al.*, 2014), the source of one of the datasets used here, and it is able to closely match the dynamics of the adult population of bees, but it underestimates the mite population in all treatments (DeGrandi-Hoffman et al. (DeGrandi-Hoffman *et al.*, 2014) Figure 3 in DeGrandi-Hoffman et al.).

It is not enough to simply have a model which includes ecologically relevant processes, however. These models ideally need to be validated against multiple independent empirical datasets (i.e. not used to calibrate or parameterise the model) to be reliably used in risk assessment. It is especially desirable that these include datasets generated under different conditions to test the reliability of the model outside the conditions it was parameterised for (Rykiel, 1996; Augusiak *et al.*, 2014; EFSA PPR Panel (EFSA Panel on Plant Protection Products and their residues), 2014).

In this study we compared the results of the BEEHAVE model(Becher *et al.*, 2014) (Available at <http://www.beehave-model.net>) to empirical results specifically focussing on the BEEHAVE model's ability to capture the behaviour of the varroa mite populations, as well as the effect of the mite infestation on the colony. The BEEHAVE model is a complex model of a honeybee colony, encompassing a large number of processes. (for details see chapter 1, page 46-) The BEEHAVE model provides sophisticated colony dynamics, with many feedback loops present reflecting mechanisms in the real colony that can show knock-on effects from impacts to one aspect of colony dynamics to many others. A review of the model by EFSA (Residues), 2015) found that the varroa in the model had little impact on colony development and suggested that the model underestimated the effect of varroa infestation. Here, we

therefore further explore the emergent behaviour of the varroa module of the BEEHAVE model when set up to match empirical studies.

In addition to the BEEHAVE model as it is published, we made four alterations to the model to explore how they would impact the bee and mite dynamics and if any lead to a better fit of the model to the empirical data. These alterations were: altering the mite reproduction rate (MRM), the impact mites themselves have on the bees in terms of mite-induced mortality (MIM), whether the mite population is subject to a global density dependence (GDD), and changing the egg-laying procedure to better suit the model to the USA climate. We used two datasets, one from the UK (BEEHAVE model was originally created to run for this climate) and one from the western United States of America, with a much different climate to establish whether the model can be localised to different climates worldwide.

5.3 Methods and model

5.3.1 The datasets

Two datasets were used in this investigation: i) a dataset from the UK, providing colony and mite development throughout a year and into the following year; and 2) a dataset from the USA providing colony and mite development in a climate for which the model

was not developed, allowing us to examine the robustness of the model in differing conditions.

5.3.1.1 UK

We used data from a study at Rothamsted Research (Hertfordshire, UK), collected between May 2011 and April 2012 by Kennedy et al. (unpublished). Material from 26 honeybee colonies was collected to make 20 test colonies, situated in apiaries in a semi-rural habitat. These colonies were assessed for varroa load and separated into two treatments, low (≤ 2 mites / 300 bees) and high varroa (≥ 7 mites / 300 bees), and these two were then further separated by taking half of the colonies in each treatment and restricting the foraging time available by shutting the colonies off from the environment from dusk to midday 4 days a week from 6th June to 26th August, thus providing free and restricted foraging treatments. In total, therefore, there are 4 treatments, free foraging-high varroa, free foraging-low varroa, restricted foraging-high varroa, and restricted foraging – low varroa. The data of most interest are the number of adult bees in the colony (assessed by counting the number of 2 cm x 2 cm grid squares wholly or partly covered in adult bees on each side of each comb, with the method being verified against counts of actual numbers from digital photographs) and the varroa infestation (assessed by using an icing sugar roll method, taking approximately 300 adult bees into a Kilner jar with a mesh lid, and shaking the bees in icing sugar, dislodging the phoretic mites and causing them to fall out of the mesh lid, enabling them to be counted) (Macedo, Wu and Ellis, 2002). These values were calculated at multiple points in the year (Adult bees – 4 weekly intervals from 1st June to 21st September and then on either 29th March or 2nd April the following year; Varroa

– between 6th -9th June, 2nd-3rd August and 26th-28th September and then again in April the following year). The initial mite population in the model was established from data on the average mite drop rate of the empirical colonies over 9 days, using the BeeBase varroa calculator (<http://www.nationalbeeunit.com/public/BeeDiseases/varroaCalculator.cfm>) to give some estimate of the actual mite population in the colony at that time, as no sugar shake estimate was taken at the start (Table 5-1).

5.3.1.2 USA †²

The data collected for the study by DeGrandi-Hoffman et al. (DeGrandi-Hoffman *et al.*, 2014) were used. This is a dataset collected in Stonyford, California, USA, in 2011. Twenty-five colonies were started from packages containing around 9000 bees, to which a queen was added. Each of the colonies was assigned to one of five miticide treatment scenarios, three of which are used in this study: 1) the control scenario, in which no treatment was applied; 2) the June treatment scenario, in which a varroa treatment (miticide) was applied in June and; 3) the Fall treatment scenario in which a varroa treatment was applied in both August and October. The two scenarios we did not test were the scenario with both a June and Fall treatment, and the scenario with just a treatment while the bees were still in the package. The treatment in this case, is taken to be the same as reported to be used in the modelled scenarios by DeGrandi-Hoffman et al. (DeGrandi-Hoffman *et al.*, 2014): that is a miticide resulting in 50%

† Empirical data in this chapter taken from raw data provided directly by G DeGrandi-Hoffmann, rather than interpolating from DeGrandi-Hoffman(2014) figures.

mortality of phoretic-phase varroa mites each day for 7 days after treatment (an efficacy value taken from DeGrandi-Hoffman et al. (DeGrandi-Hoffman *et al.*, 2012) a study on the same miticidal agent). As was the case for the UK data, we are interested in the number of adult bees in the colony (assessed by taking the number of frames covered in adult bees, including estimations of fractional frame coverage if necessary and multiplying that by 2506, an estimate of the number of adult bees on a full frame(DeGrandi-Hoffman *et al.*, 2014) (standard deep Langstroth hive)) and the varroa infestation (Table 5-1) (assessed using a sugar shake method similar to that used with the UK data, however in this case the infestation is measured as mites per 340 bees.)

UK	Average Varroa	Initial
Low Varroa, Free Foraging		20
Low Varroa, Res. Foraging		50
High Varroa, Free Foraging		290
High Varroa, Res. Foraging		390
US	Average Varroa / 340 bees	Initial
Control		13.8
June		16.2
Fall		2.2

Table 5-1

A table showing the initial mite levels in the 4 treatments for the UK data, calculated from the average daily mite drop rate using the BeeBase varroa calculator (<http://www.nationalbeeunit.com/public/BeeDiseases/varroaCalculator.cfm>) and the

initial mites per 340 bees measured for the 3 treatments for the USA data used in this study, found using a sugar shake method.

5.3.2 Adaptation of the BEEHAVE model to a Californian climate

The BEEHAVE model was originally developed for temperate climates, including weather from the UK and Germany by default (Becher et al 2014). The weather in the BEEHAVE model affects the amount of time available for the foragers to forage in the landscape. From empirical data, this can be calculated as the number of hours of sunlight on days in which the maximum temperature reaches a certain threshold value (by default 15°C). When using weather data from Stonyford, CA, USA, the location in which the USA empirical data were collected, the BEEHAVE model colony quickly dies. This is due to the amount of foraging taking place early in the year, due to good weather early in the year, as the threshold temperature for foraging to begin is set for a temperate climate. Over the winter period the majority of the adult bees in the model are foragers (as discussed in chapter 2) since all In-hive bees become ‘foragers’ at a certain age. In the over winter period, there is little to no foraging and, as such, there is a low level of mortality for foragers. However, if there is high foraging activity but no resources the forager loss is high. Since much of the overwintering population, vital for building the colony up in the following year, are foragers, any disruption to this group significantly impacts the colony. To counter this, we altered the egg-laying in the model, to ensure that the colony was producing the offspring required to maintain itself, as foragers begin foraging earlier than in the default BEEHAVE model. We used the

egg laying rate procedure from the model presented in DeGrandi-Hoffman & Curry (DeGrandi-Hoffman and Curry, 2004), which consists of expressions for four terms on day t (D_t - Degree Days, H_t - Daylight Hours, F - Number of foragers, Q_t – Number of days the queen has been laying eggs, E - Max possible eggs laid). These four expressions are multiplied together to give the number of eggs laid on day t .

Expression 1: $-0.0006 * D_t + 0.05 * D_t - 0.021$)

Expression 2: $-0.0262 * H_t + 0.809 * H_t - 5.15$

Expression 3: $\log_{10}[F + 1] * 0.672$

Expression 4: $E - 0.0027 * Q_t + 0.395 * Q_t$

In the BEEHAVE model the development of brood is already dependent on food availability in the colony, which itself is dependent on the foraging force, so we remove expression 3 from the procedure, to avoid imposing the effect of a small foraging force on egg laying twice. When this egg laying rate is used, as opposed to the BEEHAVE model default, whilst using the USA weather data, the colony grows healthily.

5.3.3 Simulations

For all simulations, 20 replicates were run. For each of the 20 replicates, the random number generator was seeded with a different value, this leads the random numbers

created by the generator to be different for each replicate, giving some variability to the results. The virus in the model is DWV as default in the BEEHAVE model (The version used in the model was “BEEHAVE_BeeMapp2016.nlogo” as available at time of writing - November 2016). None of the beekeeping options in the BEEHAVE model were included in the simulations to match the empirical procedures.

5.3.3.1 UK data

For comparison with the data from the UK, four scenarios were run, corresponding to the 4 treatments in the field experiment. These were simulating high and low varroa colonies in landscapes with free and restricted foraging (foraging time was halved relative to the default on each day between 6th June and 26th August), as calculated from empirical data. (Free foraging: high varroa – 290 mites, low varroa – 20 mites; Restricted foraging: high varroa – 290 mites, low varroa – 50 mites). The modelled colonies were set up with 8500 bees, matching the colonies in the field study, but were set up around 3 weeks earlier (May 1st) than the empirical colonies (May 20th – 25th) to give time for the virtual colonies to lay brood and gather food so that they were of similar structure at the start of the model “experiment”. The weather data used in the model were taken from the meteorological station near the field experiment at Rothamsted Research, Hertfordshire, UK in 2011. The landscape in the model consisted of four food patches, a background patch and three crops in flower for short periods, to provide a simplification of the landscape encountered by the colony, with multiple patches available.

5.3.3.2 USA data

For comparison with the data from the USA, three scenarios were run: one with no miticide treatment, one with a 7-day, 50% reduction of phoretic mites starting on the 22nd June, and one with a 7-day, 50% reduction of phoretic mites starting on both the 4th August and again on the 10th October (equivalent to 'Fall' treatment). Each of the colonies started on the 1st May, with 9000 adult bees as in the empirical study. Weather data from a meteorological station close to the study site in 2011 were used in the model. As we have no data on the landscape in the area where the empirical study took place, but we know that the empirical colonies were fed with a feeder, a simple landscape was used to present the colony with constant food. This consisted of two food patches, one at 500m and one at 1.5 km from the colony, both providing 20l of 1.5M sugar concentration nectar and 1kg of pollen each day, chosen to ensure the BEEHAVE colony was provided with sufficient protein and carbohydrate to survive.

5.3.4 Modifications to the varroa module

The BEEHAVE model is thought to underestimate the impact of the Varroa mite (EFSA Panel on Plant Protection Products and their Residues (PPR), 2015), and, when, using a mite reproduction model with little density dependence leads to a fast, dramatic increase in the mite population that became unrealistic. To attempt to achieve better pattern matching with the empirical data, we therefore alter the model to i) have the mites themselves directly affect the bees (MIM), ii) evaluate how the two mite reproduction models (MRMs) affect the mite population, iii) apply a global density dependence (GDD) to the mite population.

5.3.4.1 Mite-Induced Mortality (MIM)

There is evidence that the number of mites in a cell negatively affects the lifespan of adult worker bees (DeGrandi-Hoffman and Curry, 2004; Annoscia, Del Piccolo and Nazzi, 2012). This could be due to an increase in the viral load delivered to the individual bee, as the number of individuals showing characteristic symptoms of DWV increased with infestation (Annoscia, Del Piccolo and Nazzi, 2012) or a number of physiological effects found from infestation independent of virus (Annoscia, Del Piccolo and Nazzi, 2012). We could not find data on the exact relationship between the infestation of a pupa and the resultant adult bee mortality per day, and as such an arbitrary estimated effect was implemented. To create a general impact on health of individual bees, the model was modified to kill a certain percent of pupae upon eclosion, based on the level of mite infestation in their cell whilst developing. When a pupal cohort emerges from their cells, the number of pupae in the cohort N was reduced to:

$$N = N * (0.1 * I)$$

Where I is the average number of mites across all the cells from which pupae are emerging in this time step.

5.3.4.2 Mite Reproduction Model (MRM)

The mite reproduction model (MRM) corresponds to the maximum number of offspring per mother mite and the density dependence effect of number of mites within the larval cell on the actual number of offspring produced. Two MRMs were used in this study:

one based on data presented in Martin(Martin, 1998) allowing up to 6 mites per worker cell but applying a strong density dependent effect; and one based on Fuchs & Langenback (1989)(Fuchs and Langenbach, 1989) allowing 9 mites per worker cell with less of a density dependent effect. Both are defined in the BEEHAVE model by the maximum number of offspring a single mother mite can produce within the cell of a developing pupa (a function of the, sex (and therefore ploidy) of the developing pupa: Male(1) or Female (2)), and a density dependence factor within the cell itself (a function of the gender of the developing pupa, and the number of mites in the cell) giving a total number of offspring of:

$$\text{Total Offspring} = \text{Maximum possible offspring} * \text{density dependence factor}$$

5.3.4.3 Global Density Dependence (GDD)

Currently density dependence of the mites in BEEHAVE is controlled by local density dependence of the mites within the cell. In certain situations, this keeps the population in check, but in some cases, the mite infestation can grow very large, potentially unrealistically. In reality there will be a physical limit on the number of mites able to exist within a hive, as there is a limited number of bees to support the mite population. To counter this possibility, we implemented a global density dependence, ecologically corresponding to the fact that there is a limit to the physical number of mites that can be supported within a hive, either due to a lack of cells in which to reproduce (itself

governed by the local density dependence) or due to a lack of individual bees from which to feed. To implement this, we used the function as proposed in Ratti et al. (Ratti, Kevan and Eberl, 2012) multiplying the maximum number of offspring mites possible by:

$$DensityDependence = 1 - \frac{Total\ Mites}{\alpha * (Total\ Adult\ Bees + Total\ Brood)}$$

(where α is a factor determining the linearity (or non-linearity) of the relationship between mites and bees, the number of mites an individual bee can support)

5.3.5 Output

To assess how well the BEEHAVE model matched the empirical data, we measured two outputs emerging from the model simulations that were also measured in the empirical studies as indicators of colony growth and health. These were the number of adult bees in the colony on each day and the mite infestation level of the colony. The infestation level of the colony is calculated as number of phoretic mites divided by the number of adult bees giving the number of mites per adult bee. This value is then multiplied by the number of bees estimated to have been used in the ‘sugar shake’ infestation estimation technique: either 300 for Kennedy et al. (unpublished) UK data, or 340 for DeGrandi-Hoffman et al. (2014) USA data

5.3.6 Analysis

For the UK data, counts of the adult bees were made at 6 time points during the experiment, and varroa infestation of the colony was measured at 4 time points. Using

the mean value at each time point for the empirical data and the mean value at the equivalent time points of the model simulation data we have calculated the regression line of empirical data vs model results for both the number of adult bees in the colony and the varroa infestation. These give a quantitative description of the fit of the model to the data across the year. If the slope and intercept of the calculated regression line are close to 1 and 0 respectively, the modelled and empirical data are closely matched and, the R^2 value shows how much of the variability of the data the model explains. As we only have 4 or 6 data points, this is not intended to be a hypothesis test, but purely a descriptive exercise to provide a quantitative index.

As the USA data has only 2 time points at which the adult population was measured, the regression analysis was not carried out for this data, and qualitative assessment of the model results is presented instead.

5.4 Results

5.4.1 UK data

5.4.1.1 Adult bees

The BEEHAVE model matched the adult bee population of the empirical data quite well (Figure 5-1), for all four of the empirical scenarios (High and low varroa, free and restricted foraging), in its default setting (No GDD, No MIM, Martin MRM) (Figure 5-1B,D,F&H, orange line) with respect to the pattern over the year of the experiment. In some cases however, most obviously in the low varroa scenarios, the model colony grew less quickly than the empirical colony, leading to the model on individual days being ~10,000 bees lower than the empirical data (Figure 5-1), but displaying a similar

pattern. There was little impact on the degree to which the model matched the data on the implementation of a mite imposed mortality (MIM) (Figure 5-1, blue lines), at least during the single year comparison. Similarly, there was little impact on the colony from the application of a global density dependence (GDD) on the mite populations, with the main difference being that in the high varroa, restricted foraging scenario (Figure 5-1H), a colony did not survive in the model without GDD but all colonies survived when GDD was present. This was the only colony that died during the model simulations. In the empirical data, however, four colonies died over-winter in the high varroa, restricted foraging scenario and two died over-winter in each the high varroa, free foraging and low varroa, restricted foraging scenarios. The reproduction model of the mites (MRM) also had little impact on the adult bee population over the course of the year.

In both low varroa scenarios, the model matched the maximum size of the colony well, but grew up to that maximum level slower than for the empirical colony, regardless of any alterations (Figure 5-1 Panels C,D,G,H,K,L,O&P) This is made more clear when looking at the formula for the regression line calculated from the two sets of data (Figure 5-2) (mean of empirical data vs mean of simulated data). In the free foraging scenario (20 initial mites) the slope was relatively low (0.83, Table 5-2), and the intercept was close to 0 (-704.64, Table 5-2), implying that the modelled colony was, in general, smaller than the empirical colony and that this difference was more noticeable at larger colony sizes. For the reduced foraging scenario (50 initial mites), the slopes were slightly above 1 (1.11), but the intercept was much lower (-2864.54)

implying that the modelled colony had a larger peak, and, when it grew, it grew more quickly but was in general smaller than the empirical colony.

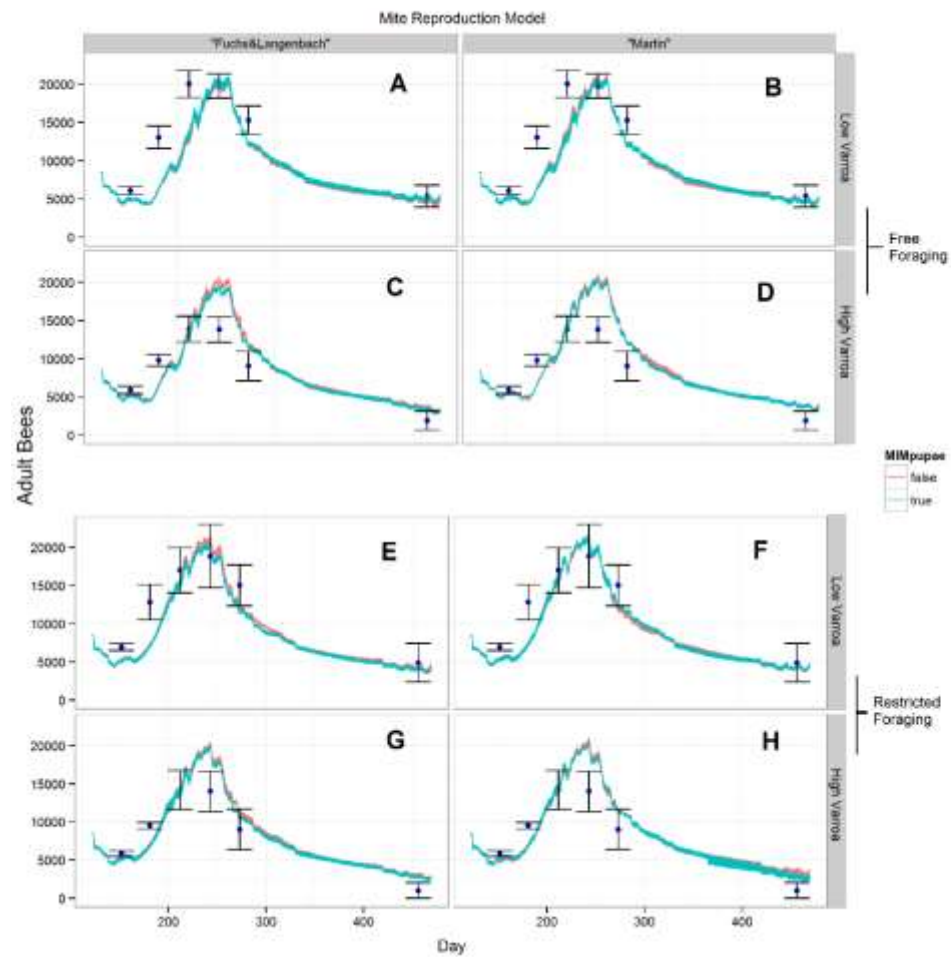
In both high varroa scenarios, the modelled colonies had a much higher peak than the empirical colonies. This is shown in the regression line, with the intercept being relatively close to 0 (free foraging -81.05, reduced foraging – 105.94) but the slope being larger than 1 (free foraging 1.16, reduced foraging 1.15). The empirical data showed higher variability in the reduced foraging scenarios, this effect was not shown in the model results for any of the scenarios.

Mite Reproduction Model	MIM	DD	FALSE			TRUE		
		Initial varroa.	Slope	Intercept	r-squared	Slope	Intercept	r-squared
Martin	TRUE	Low	0.83	-704.64	0.74	0.82	-627.36	0.74
Martin	TRUE	High	1.16	-81.05	0.74	1.08	258.43	0.7
Martin	TRUE	Low	1.11	2864.54	0.85	1.07	2626.02	0.86
Martin	TRUE	High	1.15	-105.94	0.79	1.1	624.15	0.74
Martin	FALSE	Low	0.85	-790.42	0.76	0.83	-438.14	0.73
Martin	FALSE	High	1.19	-230.59	0.74	1.09	320.38	0.71
Martin	FALSE	Low	1.09	2795.92	0.84	1.07	2602.81	0.83
Martin	FALSE	High	1.11	321	0.74	1.13	448.27	0.74
Fuchs & Langenbach	TRUE	Low	0.84	-629.3	0.75	0.87	-975.97	0.76
Fuchs & Langenbach	TRUE	High	1.11	-261.5	0.73	1.14	-220.94	0.71
Fuchs & Langenbach	TRUE	Low	1.06	2692.07	0.85	1.04	2350.19	0.85
Fuchs & Langenbach	TRUE	High	1.11	-117.39	0.79	1.1	366.74	0.76
Fuchs & Langenbach	FALSE	Low	0.82	-675.43	0.75	0.84	-843.36	0.77
Fuchs & Langenbach	FALSE	High	1.15	-258.77	0.72	1.13	-67.34	0.71
Fuchs & Langenbach	FALSE	Low	1.1	2971.89	0.84	1.06	2624.58	0.85
Fuchs & Langenbach	FALSE	High	1.12	63.59	0.78	1.13	446.93	0.77

Table 5-2

*The slope, intercept and r-squared value of the linear regression lines calculated for the results of the model simulations against the **UK** empirical data for the **adult bee population**. Corresponds to Figure 2*

No Global Density Dependence



Global Density Dependence

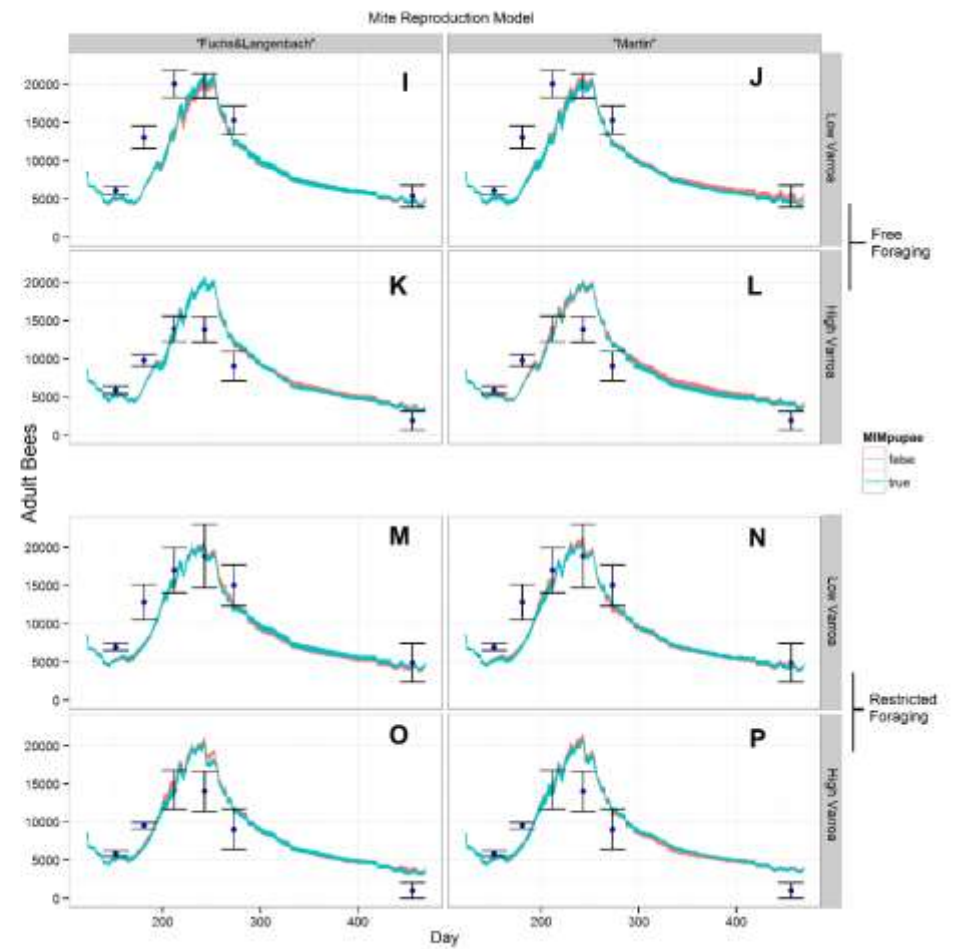
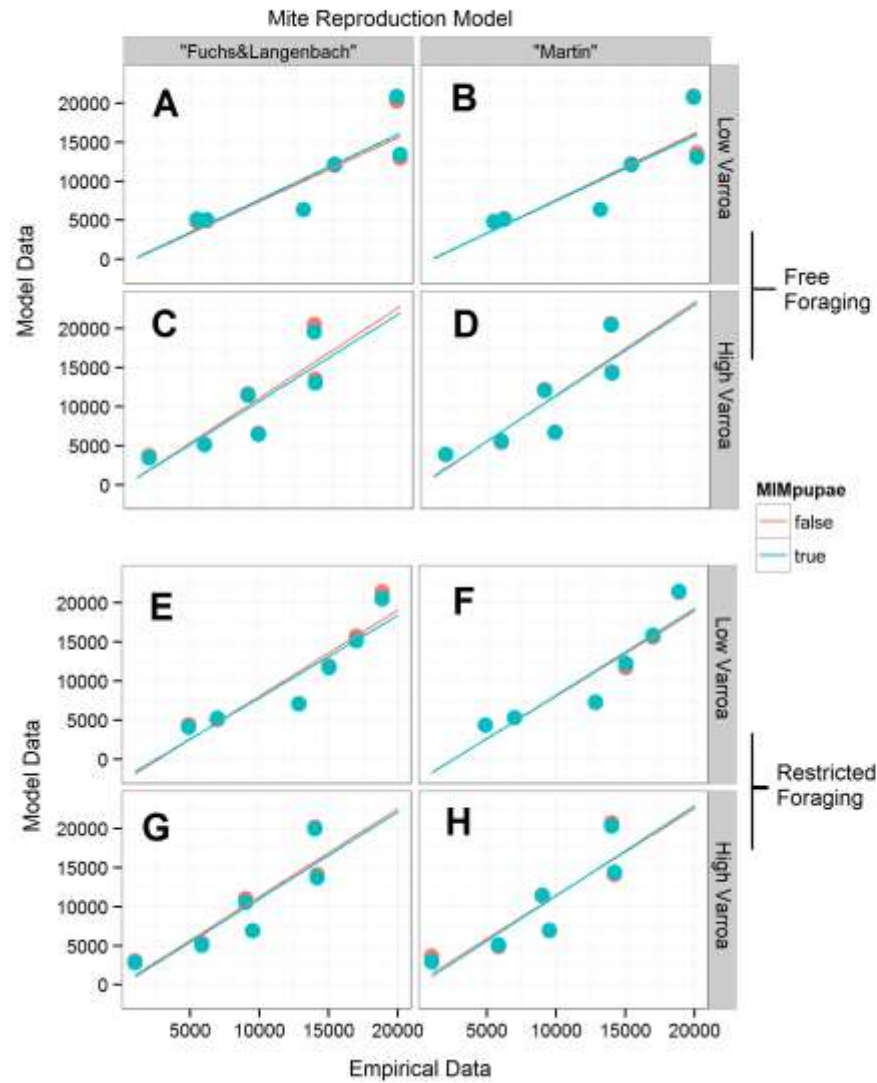


Figure 5-1

*The model results ($n = 20$) and empirical data of the **number of adult bees in the colonies for the UK**. The lines show the model results as the mean \pm the standard error, with the blue lines showing the results in the presence of the MIM and the orange line showing the results in the absence of MIM, where different. The blue points show the mean of the empirical data and the error bars show the standard error. The graphs on the left-hand side show simulations in which there was no GDD applied and the graphs on the right show simulations in which there was a GDD applied. The columns of graphs show the two MRMs used and the rows show the four initial mite populations (20 and 290 as the low and high varroa scenarios in the free foraging treatment respectively and 50 and 390 as the low and high varroa scenarios in the restricted foraging scenarios respectively).*

No Global Density Dependence



Global Density Dependence

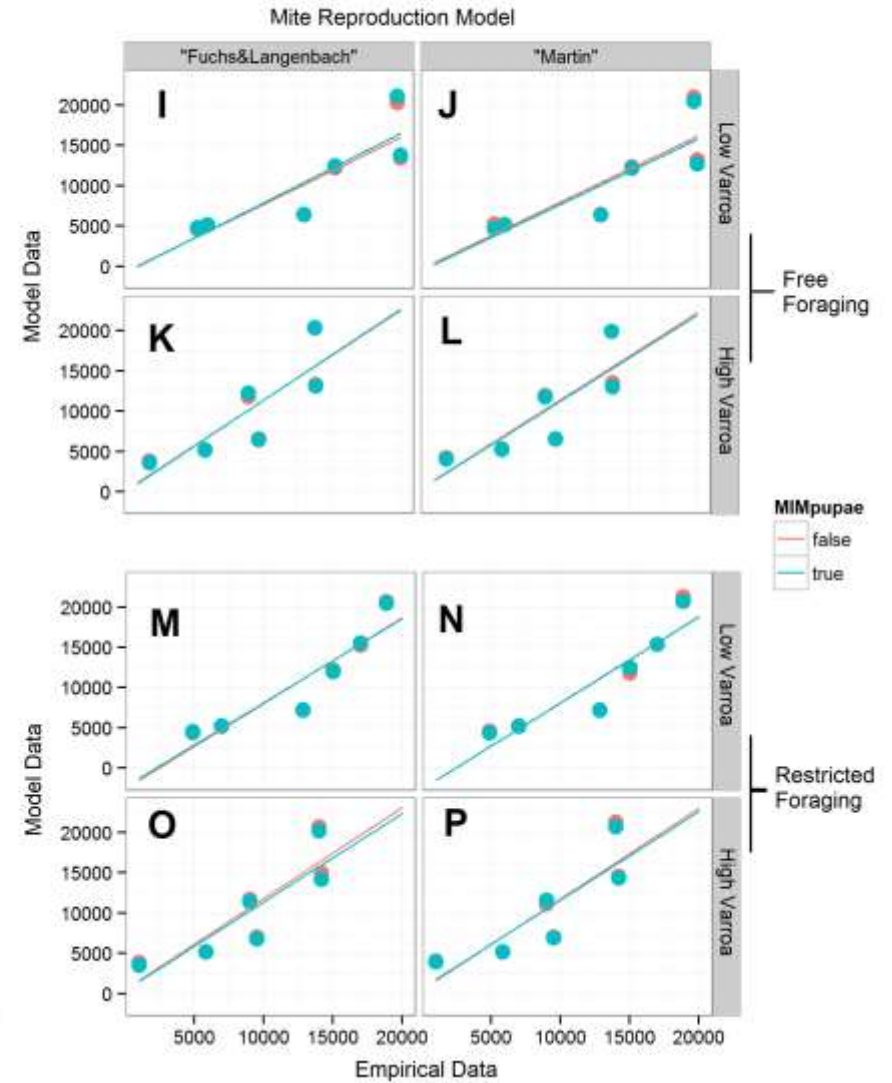


Figure 5-2

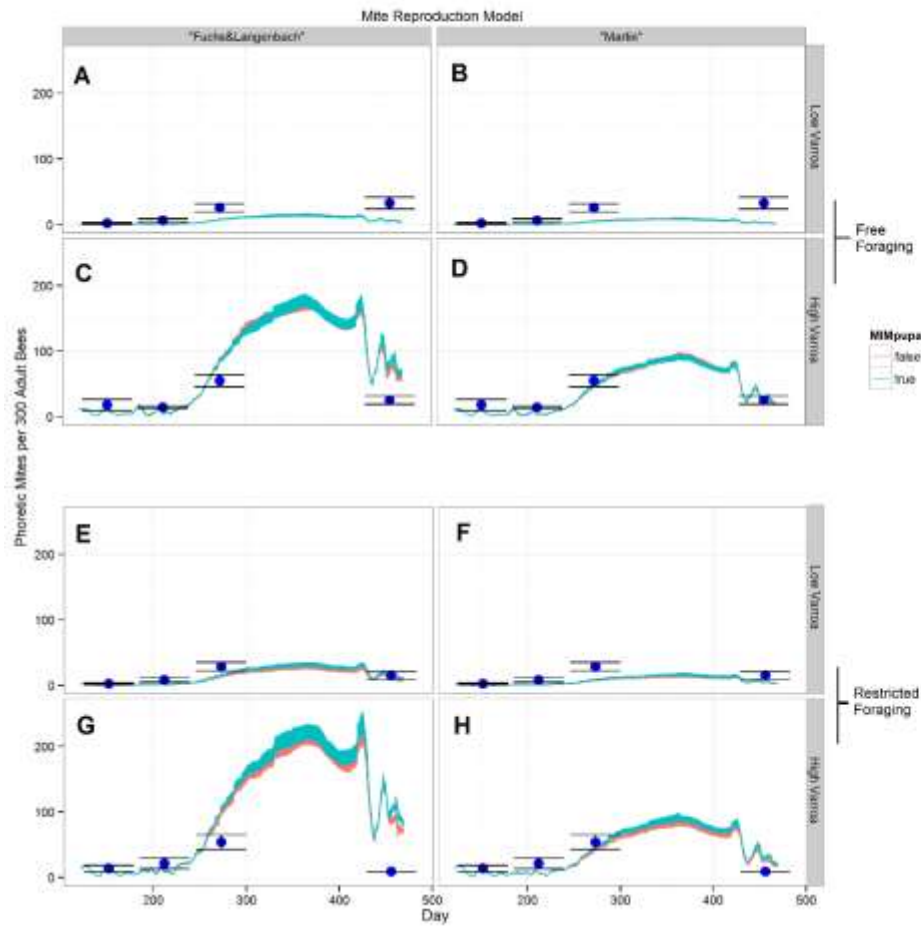
*The model results ($n = 20$) plotted against the empirical data for the **number of adult bees in the colony in UK** with linear regression lines. The blue points and lines show the results in the presence of the MIM and the orange points and line showing the results in the absence of the MIM, where different the graphs on the left-hand side show simulations in which there was no GDD applied and the graphs on the right show simulations in which there was a GDD applied. The columns of the graphs show the two MRM used and the rows show the four initial mite populations (20 and 290 as the low and high varroa scenarios in the free foraging treatment respectively and 50 and 390 as the low and high varroa scenarios in the restricted foraging scenarios respectively).*

5.4.1.2 Mite Infestation

The infestation level of the modelled colony (Fig 5-3) was more varied between the scenarios than the adult population. In the autumn of the first year, for the low varroa scenarios, the infestation rate is around 20 mites per 300 bees in the model and around 40 mites per 300 bees in the empirical data. For the high varroa scenarios the infestation rate is around 100 mites per 300 bees in the model with Fuchs and Langenbach's MRM and around 60 mites per 300 bees with Martin's MRM and the empirical colonies averaged around 60 mites per 300 bees (Figure 5-3). The presence of a GDD on the mite population (Figure 5-3 Panels I-P) reduced the infestation level of the colony, and was especially noticeable in those scenarios with a high initial varroa load. This is shown by the regression line comparing empirical to modelled data (Figure 5-4): when there was a global density dependence in the model (Figure 5-4 Panels I-P, Table 5-3). This line had a much shallower slope and a lower intercept in all scenarios, implying that in general the model results were low compared to the empirical data on the days for which we have empirical data, and that the global density dependence led to even lower infestation levels. The Martin mite reproduction (Figure 5-3 Panels B,D,F,H,J,L,N,P) rate led to lower infestation levels, whereas the Fuchs & Langenbach mite reproduction model (Figure 3 Panels A,C,E,G,I,K,M,O) led to much higher infestation levels, steeper slopes for the regression line (Figure 5-4) and, the majority of the low varroa scenarios, intercepts closer to 0. For the high varroa scenarios, the intercepts with the Fuchs & Langenbach mite reproduction model were, in general, further from zero, in both the positive and negative direction than with the

Martin mite reproduction model, but the slope of the regression line was much higher. The presence of a MIM did not have a large impact on the infestation level of the colonies in any of the scenarios (blue or orange lines, Figure 5-3). When no global density dependence (NoDD) was applied to the mite population, scenarios using the Martin mite reproduction model led to model results closer to the empirical data (Figure 5-3 Panels B,D,F&H). When a global density dependence (DD) was applied to the mite population, the Fuchs & Langenbach mite reproduction model led to model results closer to the empirical data (Figure 5-3 Panels A,C,E,G). The Martin + NoDD scenario led to a regression line slope closer to one, but an intercept further from zero, however the opposite is true for the F&L + DD scenario with shallower slopes but intercepts closer to 0 (Table 5-3).

No Global Density Dependence



Global Density Dependence

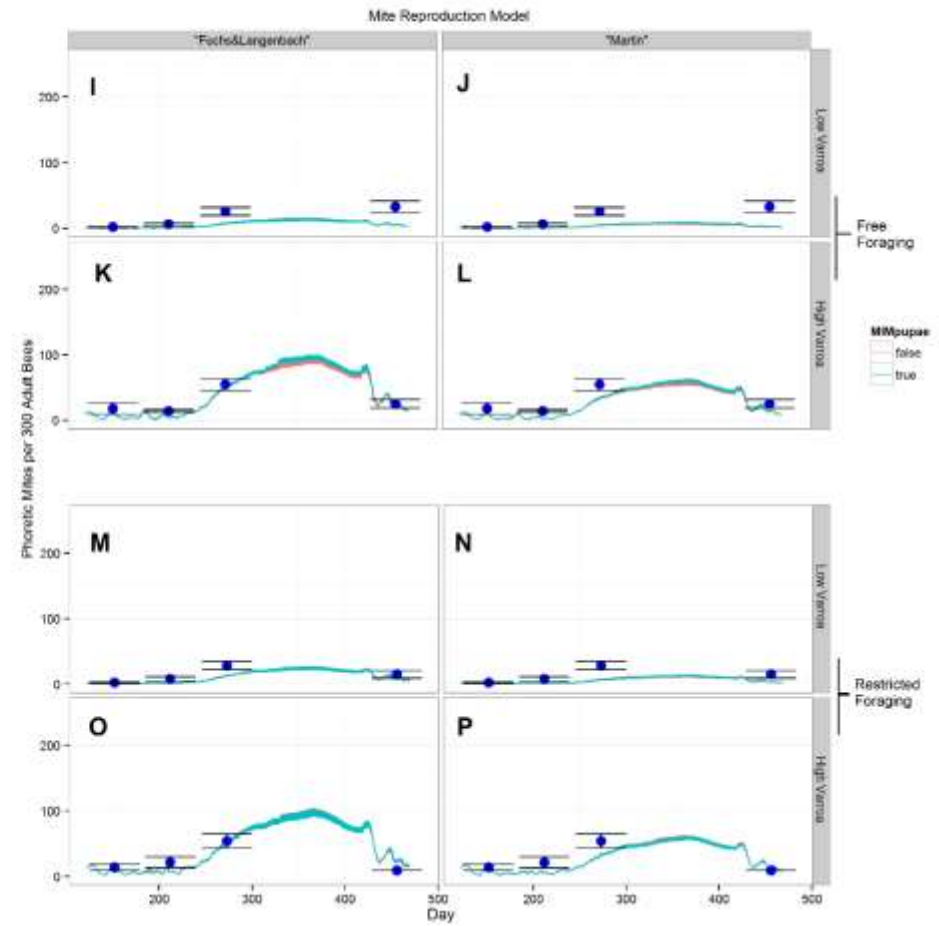
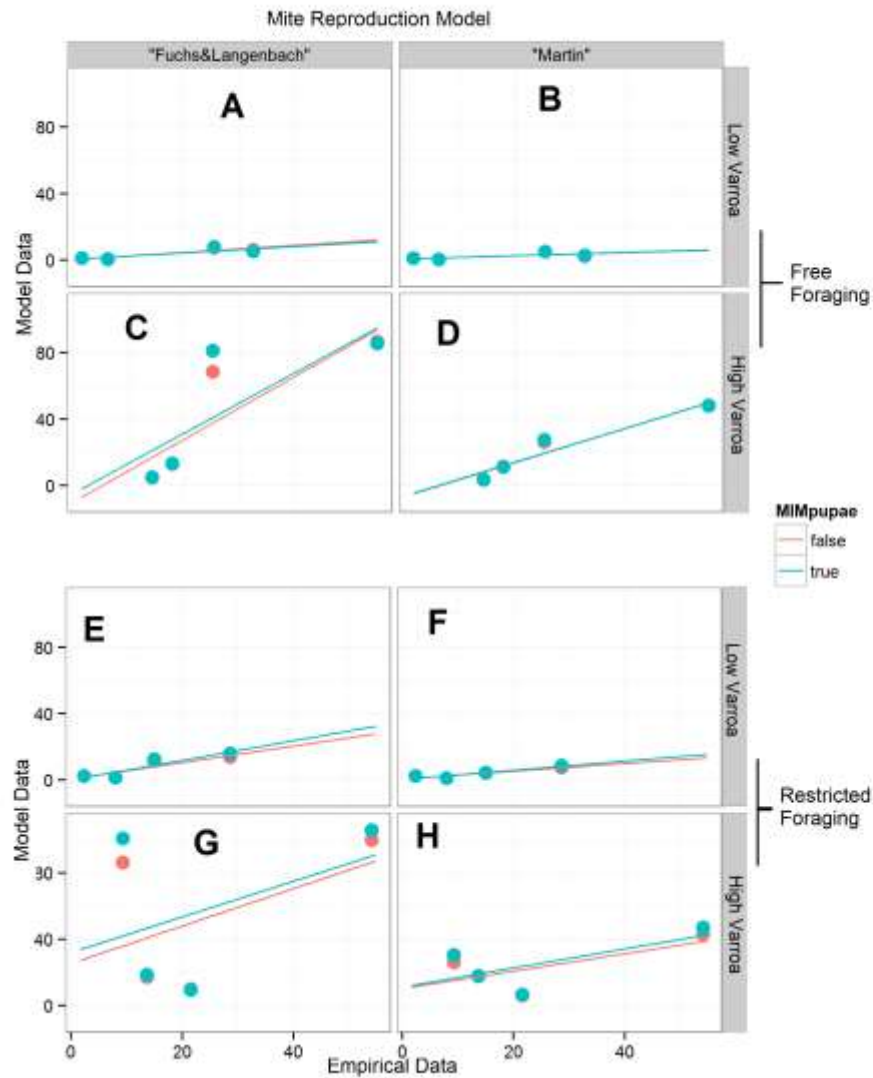


Figure 5-3

*The model results ($n = 20$) and empirical data for the **infestation level of the colonies for the UK**. The lines show the model results as the mean \pm the standard error, with the blue lines showing the results in the presence of the MIM and the orange line showing the results in the absence of the MIM, where different. The blue points show the mean of the empirical data and the error bars show the standard error. The graphs on the left-hand side show simulations in which there was no GDD applied and the graphs on the right show simulations in which there was a GDD applied. The columns of the graphs show the two MRMs used and the rows show the four initial mite populations (20 and 290 as the low and high varroa scenarios in the free foraging treatment respectively and 50 and 390 as the low and high varroa scenarios in the restricted foraging scenarios respectively).*

No Global Density Dependence



Global Density Dependence

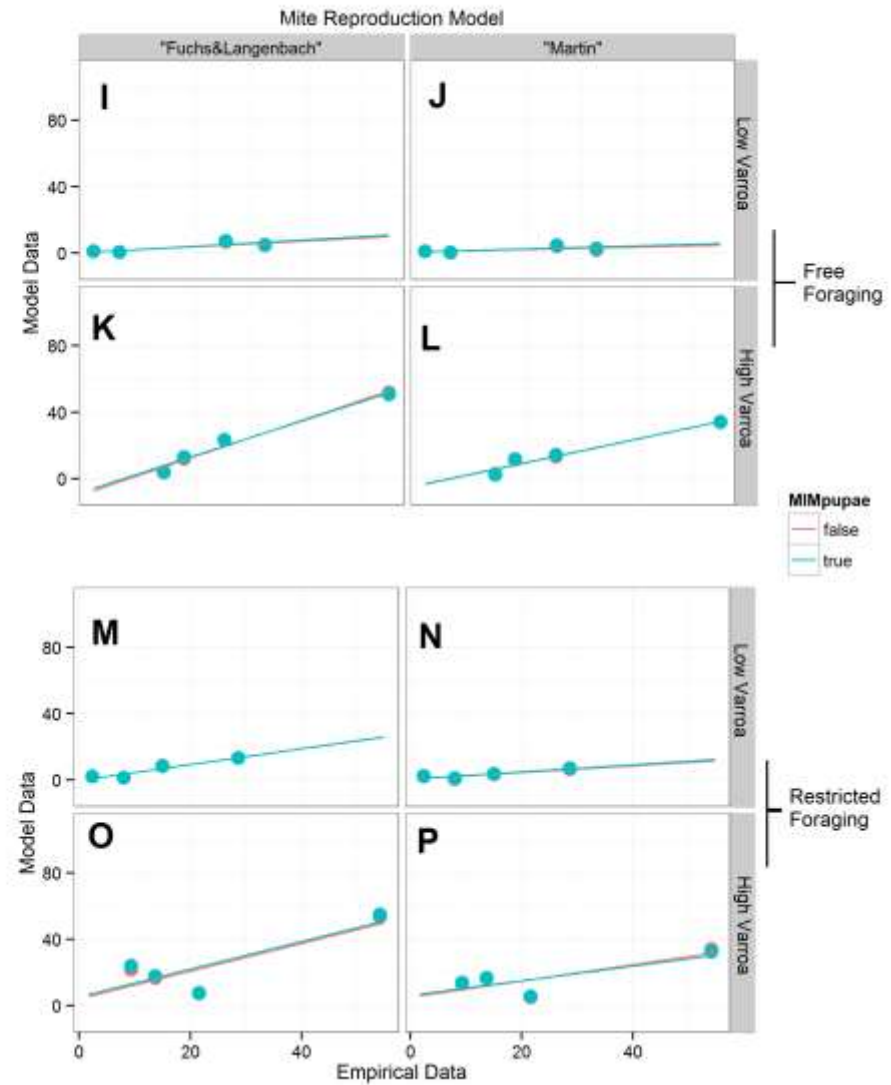


Figure 5-4

*The model results ($n = 20$) plotted against the empirical data for the **infestation level of the colony in UK** with linear regression lines. The blue points and lines show the results in the presence of the MIM and the orange points and line showing the results in the absence of the MIM, the graphs on the left-hand side show simulations in which there was no GDD applied and the graphs on the right show simulations in which there was a GDD applied. The columns of the graphs show the two MRM used and the rows show the four initial mite populations (20 and 290 as the low and high varroa scenarios in the free foraging treatment respectively and 50 and 390 as the low and high varroa scenarios in the restricted foraging scenarios respectively).*

Mite Reproduction Model	MIM	Initial varroa	No Global density dependence			Global density dependence		
			Slope	Intercept	r-squared	Slope	Intercept	r-squared
Martin	TRUE	Low	0.1	0.6	0.5	0.1	0.6	0.5
Martin	TRUE	High	1.0	-6.3	0.9	0.7	-4.3	1.0
Martin	TRUE	Low	0.3	0.4	0.9	0.2	0.6	0.8
Martin	TRUE	High	0.6	11.3	0.4	0.4	6.2	0.6
Martin	FALSE	Low	0.1	0.6	0.5	0.1	0.6	0.4
Martin	FALSE	High	1.0	-6.6	0.9	0.7	-4.5	1.0
Martin	FALSE	Low	0.2	0.6	0.9	0.2	0.5	0.8
Martin	FALSE	High	0.5	10.3	0.5	0.5	5.1	0.7
Fuchs & Langenbach	TRUE	Low	0.2	0.3	0.7	0.2	0.3	0.7
Fuchs & Langenbach	TRUE	High	1.8	-5.0	0.6	1.1	-7.6	1.0
Fuchs & Langenbach	TRUE	Low	0.6	0.1	0.8	0.5	-0.2	0.9
Fuchs & Langenbach	TRUE	High	1.1	32.0	0.2	0.8	5.4	0.7
Fuchs & Langenbach	FALSE	Low	0.2	0.2	0.8	0.2	0.3	0.7
Fuchs & Langenbach	FALSE	High	1.9	-10.1	0.7	1.1	-9.1	1.0
Fuchs & Langenbach	FALSE	Low	0.5	0.6	0.8	0.5	-0.2	0.9
Fuchs & Langenbach	FALSE	High	1.1	25.4	0.2	0.8	4.1	0.7

Table 5-3

*The slope, intercept and r-squared value of the linear regression lines calculated for the results of the model simulations against the **UK** empirical data for the **mite infestation level** of the colony. Corresponds to Figure 4.*

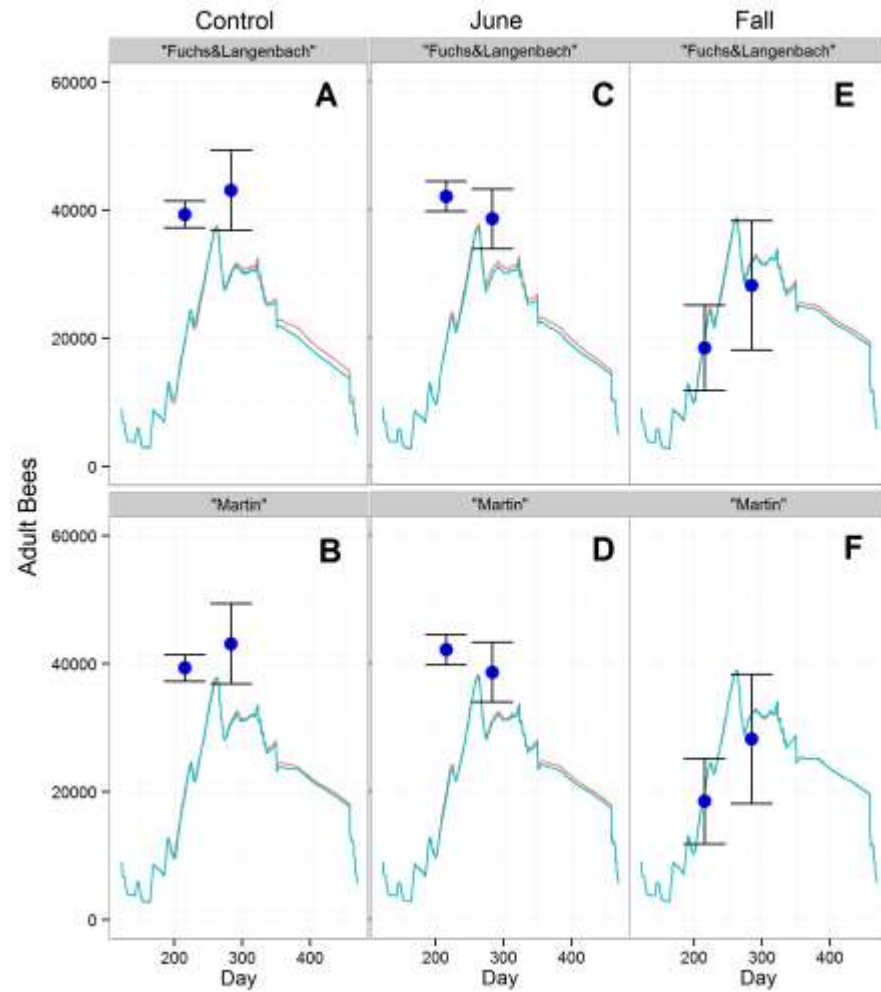
5.4.2 USA data

5.4.2.1 Adult bees

The modelled colonies reached similar colony sizes to the empirical data (Figure 5-5) (Control scenario: ~38000 vs 42000), however the modelled colony grew to that size more slowly than the empirical colonies in the Control and June treatment scenarios (Figure 5-5 Panels A-D & G-J).

As with the UK data, there was little to no impact on the adult bee population of the modelled colonies from the presence of a mite imposed mortality, the mite reproduction model used or the presence or absence of a global density dependence on the mite population. The only exception being that the presence of GDD on the mite population led to the colony in the Fall treatment scenario ending the simulation at a larger size (not declining as much). The colony developed similarly in the simulations, in all three of the treatment scenarios (Control, June treatment, Fall treatment) with little differences in the adult populations. The average modelled colony closely matched the empirical data in the Fall treatment scenario, but was smaller than the empirical data in the other two treatment scenarios.

No Global Density Dependence



Global Density Dependence

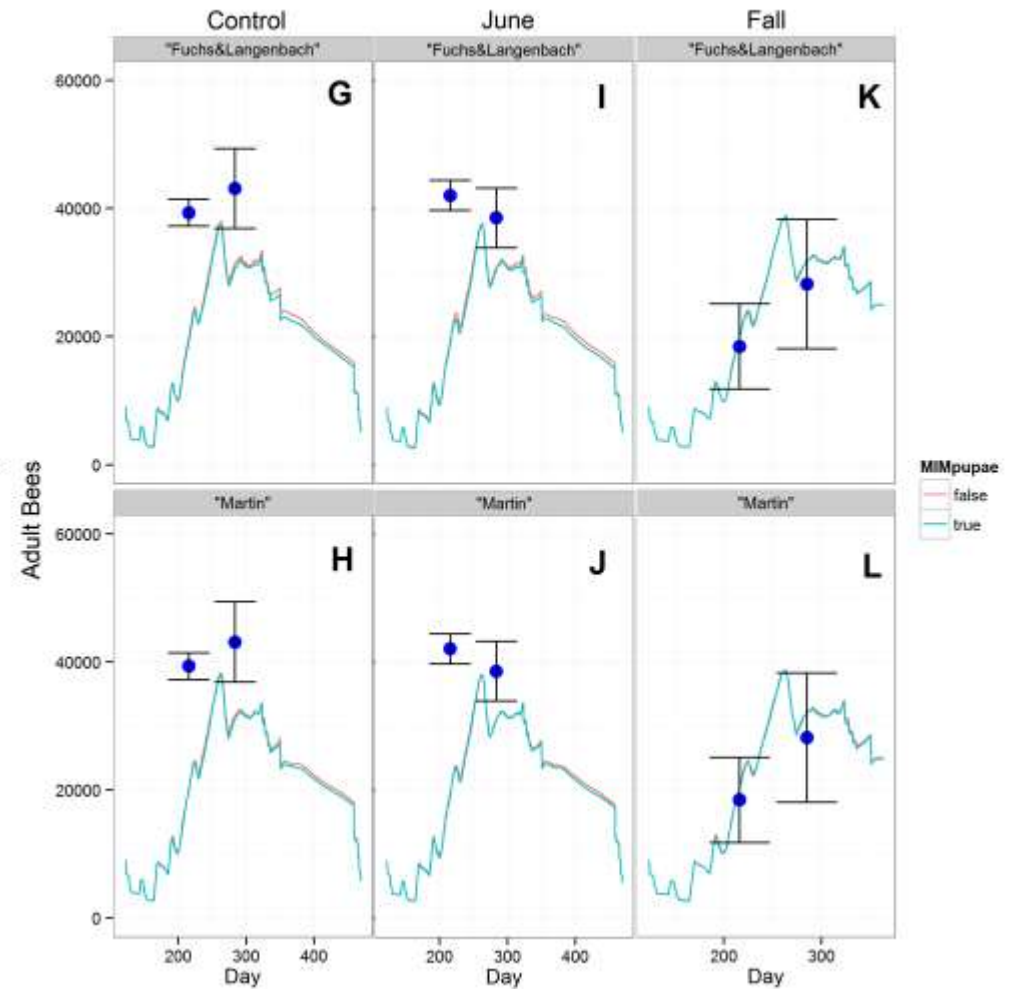
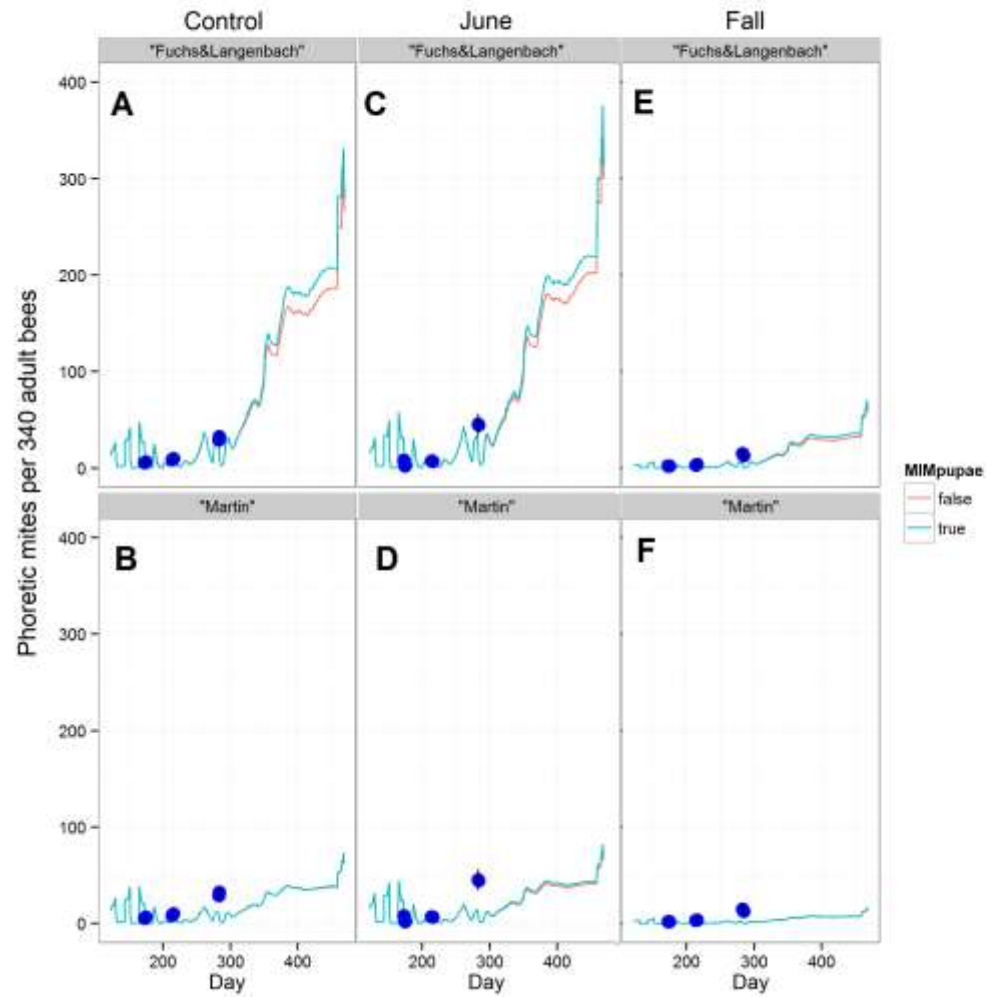


Figure 5-5

*The model results and empirical data for the **number of adult bees in the colonies for the USA**. The graphs on the left-hand side show simulations in which there was no GDD applied and the graphs on the right show simulations in which there was a GDD applied. The lines show the model results as the mean \pm the standard error, with the blue lines showing the results in the presence of the MIM and the orange line showing the results in the absence of the MIM, where different. The blue points show the mean of the empirical data and the error bars show the standard error. The rows of the graphs show the two MRM used and the columns show the three treatment scenarios.*

No Global Density Dependence



Global Density Dependence

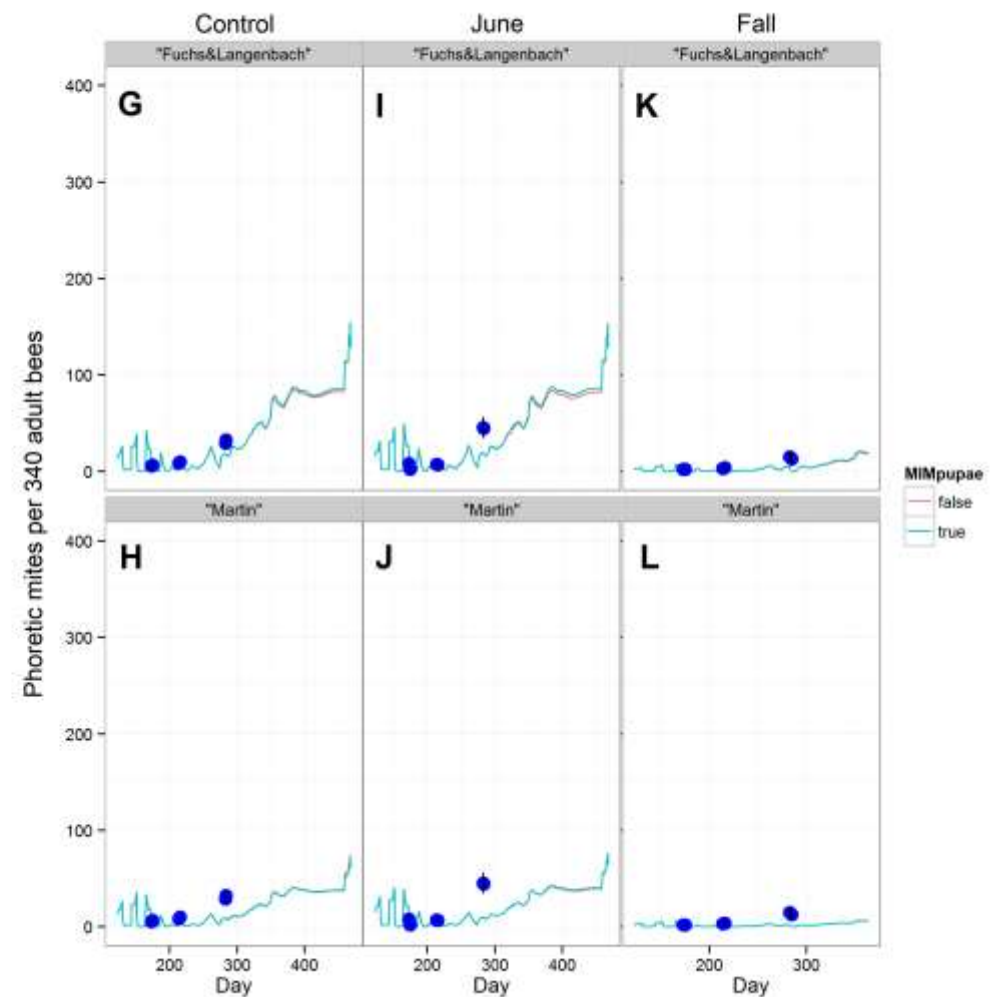


Figure 5-6

*The model results and empirical data for the **infestation level of the colonies for the USA**. The graphs on the left-hand side show simulations in which there was no GDD applied and the graphs on the right show simulations in which there was a GDD applied. The lines show the model results as the mean \pm the standard error, with the blue lines showing the results in the presence of the MIM and the orange line showing the results in the absence of the MIM, where different. The blue points show the mean of the empirical data and the error bars show the standard error. The rows of the graphs show the two MRM used and the columns show the three treatment scenarios.*

5.4.2.2 Infestation

The infestation rate of the modelled colony was similar to that found in the empirical data for the beginning of the year (Figure 5-6). Towards the end of the year, however, there is a discrepancy as the modelled colony displayed a lower infestation rate than the empirical data.

When there was no global density dependence applied to the mite population, the infestation level of the colony increases greatly towards the end of the year in scenarios in which the Fuchs & Langenbach mite reproduction model was used (Fig 5-6, panels A,C,E,G,I&K), and in these scenarios, the presence of a MIM leads to a higher infestation level (blue line) later in the simulation. When the Martin mite reproduction model was used, the infestation level remained low, with the result that the model results were lower than the empirical results on days 283 and 284 (Figure 5-5, Panels B,D,F,H,J&L).

When there was a global density dependence applied to the mite infestation (Figure 5-6, Panels G-L), the infestation level of the colony remained low throughout the simulation. For these simulations, there was little impact from the presence of a MIM. In simulations where the Martin mite reproduction model was used, the model results remained lower than the empirical data, however if the Fuchs & Langenbach model was used, then the model results were closer to the empirical results. Towards the end of the simulation, when there is the largest discrepancy in the results of the two MRMs, there are no

empirical data, making it impossible to say which MRM (in combination with other factors) gives the most realistic results.

5.5 Discussion

We have shown that the BEEHAVE model, in its default state, can closely approximate colony and mite population dynamics data from a study in the climate in which the model was created (the UK). We have also shown that with minimal modification, namely an egg-laying rate based on the weather (from DeGrandi-Hoffman & Curry(2004)(DeGrandi-Hoffman and Curry, 2004)) the BEEHAVE model is, in some cases, able to closely approximate colony and mite population dynamics from an empirical study carried out in different climatic conditions, implying that the model can be adapted to climates other than central-western Europe, which was a concern raised in the recent BEEHAVE evaluation by the European Food Security Agency (EFSA)(EFSA Panel on Plant Protection Products and their Residues (PPR), 2015). Overall the results show that the BEEHAVE model underestimates the impact of the varroa mite in the first year, agreeing with the EFSA review(EFSA Panel on Plant Protection Products and their Residues (PPR), 2015). There are modifications that may potentially increase the model's ability to simulate these real processes. Some were tested here, however this was not a comprehensive set of changes and it is unclear whether the calibration of any of these processes to one data set will cause the model to give results similarly close to any other data set.

When compared to data from a study based in the UK, the default settings of the BEEHAVE model gave results that were close to the empirical data points for both the number of adult bees in the colony and the infestation level (mites per set number of adult bees). However, the discrepancies between model and empirical data, appear to follow some patterns. When the initial mite load of the colony was low (free foraging, 20, reduced foraging, 50), the model could closely simulate the overall size of the colony, however there was a slight development delay as the modelled colony grew slower than the empirical colony, likely due to the initial conditions of the modelled colony which may need more time to build up in strength. At the same time, the infestation level of the colony is around 60% lower in the model data than in the empirical data in the autumn of the first year which, along with the fact that the adult population size was also lower, implies that the mite population was far lower than in the empirical study. At the low initial mite loads, there was little impact of using the Fuchs & Langenbach mite reproduction model (applying less of a density dependence effect) on this discrepancy. At high initial varroa loads (Free foraging 290, reduced foraging 390), the modelled colony fared much better than the empirical colonies, with a much higher adult bee population in the simulated colonies, on average. There was little impact on the adult bee population dynamics from the addition of a global density dependence on the mite population, a direct impact of the mite population on the bee population (a mite imposed mortality (MIM)) or on the mite reproduction model (MRM) used. However, there is a noticeable impact of these on the infestation level (except for the presence of an MIM). The addition of a global density

dependence on the mite population greatly reduced the infestation level of the colony, as did the use of the Martin MRM as opposed to the use of the Fuchs & Langenbach (F&L) MRM. To the extent that using the Martin MRM without a global density dependence and using the F&L MRM with a global density dependence both led to model results that are similarly close to the empirical data. This does mean, however, that if a process which reduces the adult population, such as a more severe MIM, we would predict that the mite population would then be larger than a real population. In future simulations, testing more GDD relationships, specifically non-linear relationships may yield better results.

When we compare the field data against the model results for adult bees using linear regression, all scenarios give a slope relatively close to 1 (between 0.82 and 1.16) (Figure 5-2, Table 5-2) implying that in general the number of bees in the modelled colony grew similarly to the empirical data in most cases. The regression results for the varroa infestation were much more varied (Figure 5-4, Table 5-3), with the slope of the regression line in the low varroa scenarios being much lower than in the high varroa scenarios, as the modelled colony grew much slower than the empirical colonies. This again highlights that the model captures the infestation level well in scenarios with a high initial mite load, but does not accurately model the adult bee population, so this may be misleading, and more calibration of the mite model is required.

For the USA data set, when no changes were made to the model, the colony died quickly, as there was early foraging due to the USA weather rising above the foraging threshold

early in the year, leading to a loss of the adult bees in the model from foraging mortality and the egg laying rate not sufficiently replacing those being lost whilst foraging. When the egg laying rate procedure from DeGrandi-Hoffman & Curry (DeGrandi-Hoffman and Curry, 2004) was implemented, in place of the BEEHAVE egg laying rate calculation, the colony survived for several years. This relatively small change was sufficient to lead to a modelled colony that could survive in the climate. As we only have two days in which the adult population of the empirical colonies was measured, we are unable to say with great confidence how well the model matches the data. However from the data we have, it appears that the modelled colony does not grow as large as the empirical colonies in the Control and June scenarios, which have a higher mite load at the beginning (Table 5-1), but it is much closer to the empirical data in the 'fall' treatment scenario, in which the initial mite load was lower. This is the opposite result to the UK data, in which the model overestimated the high varroa scenario adult populations.

That the initial varroa load has only a little impact on the overall adult population implies that, even with the MIM tested here, the mites in the model do not cause similar damage to the empirical mites on the same temporal scale. One reason for this with the USA data could be that the infestation level of the modelled colonies was lower than the empirical results, even with the lower adult population. This indicates that the absolute mite population is much lower in the model results than in the empirical data. In those scenarios in which the Martin MRM is used, the infestation level remains low, rising slightly at the

end of the year, whereas with the F&L MRM, a great increase in the infestation level late in the simulation. In the Control and June Treatment scenarios, when there is no global density dependence on the mites, this figure reaches and exceeds one phoretic mite per adult bee. As the infestation level in the model remained lower than the empirical data for all treatment scenarios, it is difficult to say how well the model captures varroa populations reacting to a treatment and will need to be compared with a longer study to test this.

Our results cannot be compared with the results of other varroa model predictions for several reasons: the simulations presented here were only for a year and a half, as opposed to multiple years, a different virus was used to that of Ratti et al. (2015)(Ratti, Kevan and Eberl, 2015) and the mite populations were lower than Sumpter and Martin(2004)(Sumpter and Martin, 2004). When compared to the model results in DeGrandi-Hoffman et al.(2014)(DeGrandi-Hoffman *et al.*, 2014), we find that the adult bee populations in the BEEHAVE model are lower than the empirical data at both points, whereas the DeGrandi-Hoffmann model results led to a higher average population in October. Interestingly, the BEEHAVE model captures the growth of the mite infestation level later in the year better than the model results presented in DeGrandi-Hoffman(2014)(DeGrandi-Hoffman *et al.*, 2014), although the infestation level is in most cases, lower than the empirical level.

These simulations highlight both that the BEEHAVE model can reliably match empirical data on adult bee and mite population dynamics in some situations, but at the present

time, more study would be needed in order for the varroa sub-model to be validated to the point it could act as a reliable predictive tool for the Varroa mite. We have shown that there are a number of ecologically valid alterations that could be made to the BEEHAVE model varroa module for the model results to better reflect empirical data. We were unable to successfully calibrate the alterations tested to match every situation, but were able to closely match empirical data in some situations. The next step would be to take the altered model, calibrated to the data sets used in this study and see how the calibrated model compared to other datasets with similar conditions to validate the model (Rykiel, 1996; Augusiak *et al.*, 2014), and use this model to target empirical studies to fill data gaps. One important factor not considered in this study is the viruses carried by the mite. We used a single virus with a single parameterisation for simplicity, but in the real world it is likely that mites will be delivering a cocktail of viruses to bees upon which they feed. Modelling the exact movement of viruses within the colony and how any impact from these viruses in the bees synergise and interact would be a very difficult task, and a simplification may be necessary. The MIM tested in this study had little impact on the adult population of the colony or the mite infestation. The method used here was chosen as a simple reduction in pupae based on mite infestation, as we were unable to find data on the precise impact of mites on adult bees, although Annoscia *et al.* (2012) (Annoscia, Del Piccolo and Nazzi, 2012) have good data on lifespan of adult bees from infested cells infested with 1 and 3 mites. If more data were available on individual daily mortality of infested bees, then this

would be able to be implemented into the model and it would be possible to see if the model would then more closely match empirical data.

The Varroa mite poses a serious threat to honeybee colonies, especially when considered alongside the diseases for which it is a vector (Genersch *et al.*, 2010). As such it is important that we are able to understand how it affects bee colonies and how best to treat them e.g.at what infestation level and when, especially when considering treatments that themselves harm the bees. We have shown that the BEEHAVE model has definite potential to be used as part of this research, and we would suggest that it could be especially useful to answer questions about miticide usage as miticide impacts to the mites and bees. These can be easily and reliably implemented, and this is a vital area of applied research that would benefit from use of the model.

Chapter 6 - Discussion

Many countries worldwide have been reporting high honeybee colony losses (Neumann and Carreck, 2010; Potts *et al.*, 2010; vanEngelsdorp *et al.*, 2012), although globally, stocks are increasing (Aizen and Harder, 2009; Potts *et al.*, 2016). As the honeybee contributes to pollination (Breeze *et al.*, 2011), and pollination is a highly valuable service (Gallai *et al.*, 2009), understanding these losses and how to most efficiently mitigate them is highly important.

The honeybee is subject to a large number of simultaneous stressors in the environment, such as changes to land use (Naug, 2009), parasites (Boecking and Genersch, 2008), diseases (Wilfert *et al.*, 2016) and pesticides (Johnson, 2015).

In this thesis I present results from simulations using the BEEHAVE model and a novel model that answer broad questions:

A. How does increased mortality of individuals at different life stages (a surrogate for pesticide impact) scale up to colony-level impacts? (Ch. 2 & 4)

B. How does the individual behavior of the workers affect likely exposure to pesticides?

iii) Storage behavior In the hive (Ch. 3)

iv) Foraging behavior in different landscapes (Ch. 4)

C. To what extent does the timing of a pesticide exposure affect the resultant impact on the colony? (Ch. 2, 3 & 4)

D. How well does the BEEHAVE model simulate the population dynamics and impact of the varroa mite, and is it robust to different climates? (Ch. 5)

I will first summarise the key findings of the Chapters 2-5 before entering a broader discussion of the potential practical application of these results.

Specifically, I find that simulated pesticide-induced mortality to the in-hive worker bees has a more damaging impact on the growth of the colony than the mortality of other life stages, with increased larval mortality and reduced egg-laying rate having the least impact to the colony (Chapter 2) (Rumke *et al.* 2015). This is similar to results from other models (Bromenshenk *et al.*, 1991; Schmickl and Crailsheim, 2007). The colony is sensitive to losses of foraging bees during a foraging trip (Chapters 2 & 4), this is also suggested by Henry *et al.* (Henry *et al.*, 2012), and could be a result of younger bees becoming foragers early, and as a result are less efficient (Perry *et al.*, 2015). This is important to keep in mind, as the results of the novel model (Exploring how in-hive distribution of pesticide-containing nectar can affect the exposure of individuals within the hive - Chapter 3) find that when considering exposure to pesticide through nectar consumption, assuming that the in-hive bees consume nectar containing a pesticide concentration equivalent to the total weight of pesticide brought in on that particular day in the total volume of nectar brought in on that day gives a conservative estimate of

exposure. Therefore, for risk assessment, this estimated average value could be used for the exposure of in-hive workers, and is likely to be conservative, although it may not be conservative for the larvae, especially considering the proportion of larvae reaching a threshold dose. When considering the mortality of foragers whilst out collecting food (as in Henry et al. (Henry *et al.*, 2012)), the organization of the landscape, both in complexity and where in the landscape the pesticides are located significantly affects the resultant impact on the colony (Chapter 4). A beneficial landscape, providing pesticide free forage close to the colony can offset the impact of a pesticide-treated crop further from the colony in the landscape, as can a more varied landscape with many patches for the foragers to exploit (Chapter 4), however if the pesticide in question moves in the environment, or is applied as a spray and drifting occurs nearby plants could also be contaminated. Also, a very important result is that the timing of a pesticide exposure seriously alters the colony's response (Chapters 2 & 4). The colony begins the year with the over wintering bees. Then in spring, foraging begins and egg-laying starts, the colony begins building in numbers going into summer and then, around late-summer to early-autumn, the colony reduces in size as egg-laying slows to enter the over-winter period again. If a pesticide is available early in the year, it will likely not be encountered by foragers (unless the hive is exposed directly), and will have little impact. As the colony begins to build, major reductions to the colony strength caused by a pesticide exposure event will reduce the population size in summer, reducing the colony's ability

to collect and store food for the winter and increasing resultant overwinter loss (Chapter 2).

I have also assessed the BEEHAVE model's ability to capture the population dynamics and impact of the parasitic varroa mite. The results of the simulation's show that the model does capture the dynamics of the mites, importantly the increase in mite numbers late in the year, but underestimates the impact of the mite on the bees, even with additional mite-induced stresses incorporated (Chapter 5).

6.1 Implications for pesticide risk assessment

The current guidance from the European Food Safety Authority ((Efsa), 2013b) (EFSA) asserts that a 'negligible' impact of a pesticide on the honeybee colony is between a 3.5% and a 7% reduction in colony size. This is translated to a trigger value, used in the first-tier risk calculations (exposure-toxicity-ratio or toxicity-exposure-ratio) as a limit point: If the ratio of the exposure of the bee to the pesticide to the toxicity of the pesticide is above the trigger value, then higher tier assessments are necessitated. Currently, for chronic oral exposure, the model presented by Khoury et al. (Khoury, Myerscough and Barron, 2011) (with the parameterisation as used by Henry et al. (Henry *et al.*, 2012)) is used to calculate this trigger value by using the Khoury model to find the mortality that will lead to a 7% reduction in colony size ((Efsa) 2013b, appendix M). The results presented here (Chapters 2 & 4) show that the colony response to an increase in mortality is highly dependent on timing of the pesticide exposure and the

landscape in which the exposure takes place. The Khoury model (Khoury, Myerscough and Barron, 2011) is a simplistic model and does not necessarily capture these processes. EFSA reviewed the BEEHAVE model (EFSA Panel on Plant Protection Products and their Residues (PPR), 2015) and find that BEEHAVE could be used in place of the Khoury model to calculate the relevant trigger values. The results presented in this thesis are in agreement with this. The EFSA review of the BEEHAVE model (EFSA Panel on Plant Protection Products and their Residues (PPR), 2015) suggests the modelling of residue redistribution within the hive as an expansion to any future regulatory risk assessment model, with the justification being that individual bees will feed from individual cells which will not have a uniform distribution of pesticide. The model presented in Chapter 3 (ODD in appendix 1) was designed to address this concern specifically and gives evidence that the specific modelling of the in-hive distribution of nectar is not required for the conservative estimation of the exposure of the in-hive workers to pesticides, rather assuming the adults consume nectar containing the average concentration' of a pesticide for each particular day (total weight of pesticide in the total volume of nectar), is a more conservative estimate (Figure 3-3) Larval exposure, however, as observational evidence shows that nectar and pollen are emptied from cells close to brood (Camazine, 1991), is not conservatively estimated by an average exposure. Extreme clustering of pesticide in a section of the comb can lead to a higher exposure. However the results presented in Chapter 2 imply that unless the

pesticide in question is highly toxic and/or present for an extended period, the impact on the colony may not be as extreme.

In all simulations presented in this thesis, the dissipation and degradation of pesticides has not been considered. Pesticides will break down over time in both the plant (Fantke *et al.*, 2014) and within the cells of the hive comb. Depending on the chemistry of the pesticide, there may also be movement from the stored food within the cells into the wax (Tremolada *et al.*, 2004), potentially causing negative impacts to the brood (Wu, Anelli and Sheppard, 2011). Metabolism of pesticides (Cresswell *et al.*, 2014) was not considered in the model presented in chapter 3. As the pesticide is carried by the forager, in the pollen or in water or nectar, and then as it is processed and gathered to feed larvae, it may be metabolized by the workers, reducing the pesticide content of the product. The model does not incorporate pollen or water, though, if necessary could be expanded to do so, and also does not explicitly model the creation of brood food by the nurse bees.

6.2 Implications for the mitigation of stressors

For the mitigation of potential impacts of pesticide exposure, the results of the simulations presented in this thesis suggest a number of potential avenues. Firstly, the timing of the exposure has a very large effect on the resultant response of the colony (also found by Thorbek *et al.* with regards to sublethal effects (Thorbek, Campbell and Thompson, 2016)).

Of the large scale field studies, there have been studies with exposure early in the year (April-May) finding no impact of the pesticide (Rolke *et al.*, 2016), and studies with exposure around June also finding no impact of the pesticide at the colony level (Cutler *et al.*, 2014; Rundlöf *et al.*, 2015). However these have all been focused on a single crop type, oilseed rape, treated with a neonicotinoid seed treatment. When considering a more general pesticide exposure (perhaps a foliar spray applied during the day) leading to foragers returning to the colony with pollen and nectar containing a high pesticide content, the most damaging period for this to occur is from June onwards, as this is the period when the colony is collecting food for the over-winter period. Later in the year, if there is still foraging occurring, a high mortality of the in-hive bees will severely disrupt the colonies ability to overwinter.

These results show that the landscape, its complexity and relative distance of food from the colony can exaggerate or mitigate pesticide impacts to the colony, and also modify colony health directly. These results imply that having a more varied landscape, or ensuring that the honeybee colonies are not closer to treated crops than they are to other forage sources could reduce the impacts of any potential pesticide effects.

Clermont *et al.* (Clermont *et al.*, 2015) find that high honeybee colony losses are associated with land use for industrial or recreational human use (golf courses, sports fields, campsites etc.) and that low colony losses were associated with maize fields and mixed forests, and Ricketts *et al.* (Ricketts *et al.*, 2008) find that pollinator diversity and activity reduce with distance from natural and semi-natural habitat. From the simulation

results and empirical results, it is possible to predict that ensuring that honeybees have a good, varied landscape offering food throughout the year would help reduce impacts from pesticides, but also possibly allow the colony to withstand other stressors (Thorbek *et al.*, 2016).

6.3 Modelling of varroa mites

While pesticide impacts receive much of the media attention, many field studies and meta-analyses have found the varroa mite and related effects to be a very important stressor driving colony losses (Boecking and Genersch, 2008; Genersch *et al.*, 2010; Neumann and Carreck, 2010; Kielmanowicz *et al.*, 2015). Simulations presented in this thesis show that the BEEHAVE model is capable of capturing the dynamics of the varroa mite, but calibration of the exact level of damage the mites and viruses have on the honeybee colony is required. There are a number of models of the varroa mite and its related stressors (Martin, 2001; DeGrandi-Hoffman and Curry, 2004; Sumpter and Martin, 2004; Ratti, Kevan and Eberl, 2015; Kang *et al.*, 2016), however the BEEHAVE model was conceived and designed to incorporate multiple stressors at once (Becher *et al.*, 2014). This will allow a very important question to be investigated: that is, at what level of varroa infestation is treatment with a varroacide necessary? Many varroacides used can also harm the honeybee, especially in combinations (Johnson *et al.*, 2013), and so a careful approach to treatment is important. There are already tools to advise beekeepers, such as the Beebase varroa calculator

(<http://www.nationalbeeunit.com/public/BeeDiseases/varroaCalculator.cfm>), but with the

landscape module and detailed colony dynamics (and the results of the extent to which the forage landscape affects the colony health), calculations based on the landscape in which the interested beekeeper is based (which could be at a coarse resolution based on land use in the area) and the specific treatment regime could be used to guide treatment. Results from these simulations show that the model is capable of carrying this out, with more data available for calibration.

6.4 Final conclusions & future directions

Using the BEEHAVE model and a novel model, I have shown that pesticide-stress has the potential to harm the honeybee colony, however this harm is highly dependent on the timing of exposure, the life stage targeted and the landscape quality and complexity. These results have further cemented the BEEHAVE model as a highly capable model for the risk assessment of pesticides and other stressors. I presented a novel model showing that, in the case of in-hive worker exposure, assuming an averaged exposure is sufficient for a conservative exposure estimate, contributing towards EFSA's advice on extensions to the model for it to be used in risk assessment. I have also shown that the model is able to capture varroa mite dynamics, although more calibration is needed. As further empirical data becomes available, then the BEEHAVE model can be modified and calibrated to capture the impacts of varroa mites, virus and pesticide treatments at the resolutions required for reliable prediction.

The results presented in this thesis, along with various other studies highlight a number of directions for future work. There are currently a number of knowledge gaps that, if filled would allow a more realistic modelling of the honeybee colony under stress. Firstly, for the impact of pesticides on the honeybee colony. There are, as is required for the registration of pesticides, data on the impact of individual chemicals on the individual bee up to the colony level, in terms of survival of individuals and results of monitoring the colony as a whole. These data on the dose-response relationship of a pesticide at a known level are vital for the reliable modelling of pesticide impacts onto the honeybee colony. For example, in creating a pesticide model for the BEEHAVE model, the way pesticides affect the individuals in the colony is a necessary input and the effect of these pesticides on the colony would be extremely useful to validate patterns emerging from the model. In addition to the impact of a known pesticide dose to an individual, the movement of the pesticide into the colony through the foraging dynamics and the movement within the colony, and the differential exposure of individuals depending on their life-stage or job within the hive: in other words, the exposure of individuals to pesticide applied in a realistic manner (i.e. in a treated crop as opposed to a treated feeder or pollen patty) are not as clearly understood. For the risk assessment of these chemicals, for which a conservative estimate of exposure is suitable, this is less of a problem, however from an academic perspective when the actual exposure of individuals is of interest, this would be very useful. To gather this data empirically would be difficult, however. Ideally, one would have data on the foraging behaviour of a hive

within a known landscape, and then the content of the food and water brought back into the colony along with its movement within the colony.

Secondly, due to the impact of the varroa mite on the honeybee colony, it is clear that a proper understanding of the mechanisms through which the mite and its related diseases impact individual bees and the colony is vital. It is known that mites are able to cause damage to the individuals in the absence of a viral load. For the modelling of this stressor a quantified impact would be extremely useful. A systematic study of the impact of infestation throughout development and on adult bee lifespan and efficiency (number of brood fed, nectar loads from foragers received, foraging activity compared to uninfested individuals for example) could provide some of the required data. However, once again, the logistics of performing an empirical study of this magnitude may be too complex to be practical, but building up the data to fill the knowledge gaps in a number of smaller experiments would still provide the necessary information. The BEEHAVE model has great potential, as the results presented here and other work have shown. However, this work has also highlighted priorities for inclusion in the model during future development. These would include: i) further differentiation of the adult worker bees by the role played in the hive, as this can potentially affect the pesticide exposure to the individual and the impact on the colony of losing that individual; ii) the ability to model a dose-response relationship of a pesticide on the individual life stages, including synergistic effects between pesticides; iii) pesticide application events within the landscape and the movement of the pesticides into and within the ; and finally, iv)

modelling the impact on the honeybees of the varroa mites themselves, as opposed to solely an impact from the virus.

Appendix 1 – ODD protocol for Chapter 3

Purpose

The purpose of this model was to assess how different food storage and feeding behaviors of the honeybee affect the distribution of pesticide concentration in stored nectar, and explore how different distributions of pesticides affect the proportion of individuals (brood and adult bees) which will be exposed above a theoretical threshold (set to an arbitrary level here but which could be defined based on a pesticide's toxicity). The model can then be used to assess the complexity required in introducing realistic in-hive pesticide exposure into an existing honeybee colony model (e.g. BEEHAVE (Becher *et al.*, 2014)). In particular, we set out to compare pesticide distributions as a result of the following contrasting behaviors : i) comparing multiple transfers between foragers and receivers (M) as opposed to each forager transferring nectar to a sole receiver (S); ii) comparing when receiver bees store nectar in the comb randomly (R), versus clustering (C) iii) comparing the effect of capping the nectar cells, (as a result of processing to honey) (P) versus no capping (N). We also investigate the impact of differing proportions of foragers bringing pesticide into the colony, a simplified surrogate for pesticide exposure levels in the landscape.

The model is not intended to provide accurate estimates of the absolute values of exposure or toxic effects of pesticide within the hive, rather, it is intended to explore the differences in pesticide distributions in nectar occurring from these simplified behaviors, and therefore establish the level of complexity required for a model such as BEEHAVE (Becher *et al.*, 2014; EFSA Panel on Plant Protection Products and their Residues (PPR), 2015) to ensure a conservative assessment of the risk posed by pesticides. The model simplifies feeding by the nurse bees, without modelling the production of brood food, instead having a direct transition of nectar to the larvae.

Entities, state variables and scales

Agents/individuals

The model contains three classes of agents: The cells of a single, one-sided hive comb, the bees and the forage patches. The cells of the hive comb are spatial units, implemented as 'patches' in NetLogo.

Each cell is characterized by the following state variables: 1) *patch_type*: patch contains nectar or a larva or is empty; 2) *nectar_volume_μl*: the current volume of nectar in the cell; 3) *pesticide_concentration_μg/L*: the concentration of pesticide in the cell, if the cell is a nectar cell; 4) *cell_nectar_concentration_μg/L*: the concentration of the sugar in the nectar contained in the cell;

A single nectar load is assumed to be 14 μ l, within the range reported by Huang and Seeley (2003)(Huang and Seeley, 2003)

The forage patches are characterized by the following variables: 1) *nectar_concentration_ μ g/L*: the concentration of sugar in the patch; 2) *field_pesticide_concentration_ μ g/L*: the concentration of pesticide in the patch;

There are four types of bee agents in the model: 1) foragers; 2) receivers; 3) larvae; 4) the queen. In the rest of the manuscript, ‘adults’ represent a combination of the foragers and receivers, who’s feeding requirements are assumed to be the same for simplicity. A nectar load in the model is 14 μ l(Huang and Seeley, 2003) . This is the amount carried by the adult bees and is constant.

The forager bees are characterized by the following variables: 1) *pesticide_amount_ μ g*: the amount of pesticide carried by the forager; 2) *carrying_nectar?*: a Boolean value, true if the forager is still waiting to transfer nectar to a receiver; 3) *carrying_2nd_nectar?*: a Boolean value, true if, when multiple transfer is active, the forager is waiting to transfer the second load of nectar; 4) *nectar_sugar_concentration_ μ g/L*; the concentration of sugar in the nectar load carried by the forager;

Receiver bees are characterized by the following variables: 1) *pesticide_weight_ μ g*: the amount of pesticide currently carried by the receiver; 2) *destination*: the receiver’s cell of

choice in which to deposit the carried nectar load; 3) *nectar_sugar_concentration_μg/L*: the concentration of sugar in the nectar load carried by the receiver;

Larvae are characterized by the following variables 1) *age*: the age of the individual in days; 2) *pesticide_amount_μg*: the amount of pesticide contained in the larvae; 3) *cell_choice*: the cell the larvae will be fed from.

The queen is characterized by its location on the comb, the only role of the queen in this model is creating new brood with a realistic spatial distribution.

The spatial scale of the model is set to represent a typical comb of a National bee hive (*British Standard Bee Hive Frame Dimensions*, no date) assuming a frame of 34.1 x 20.3 cm with 4.34 cells per cm². The comb consists of a grid of square cells, 80 x 40, giving 3200 cells, a reasonable estimate of the number of worker cells on one side of a frame (Camazine, 1991).

The model runs in daily time steps with the foraging, receiving and feeding processes looped to implicitly represent hourly behaviors, (e.g. foraging, receiving, storage and feeding) and others happening once per day (processing).

Units

The model keep track of pesticide and sugar as both concentrations and mass. When dealing with volumes larger than a single bee's nectar load (such as in a nectar cell or at the forage patch) the substance is stored in the model as a concentration. When being handled by an individual, i.e. in foraging, receiving, storage and feeding, the substance is stored in the model by the mass of the substance. This facilitates the calculations required when nectar is stored or removed from a large source (cell or forage patch) and allows a practical understanding of the potential exposure of individuals to the substance within the hive (individual dose received and pesticide concentration in nectar stores). For concentrations of pesticides and sugar in the model, we use weight per volume ($\mu\text{g/L}$). The mass of a substance is measured in μg and when discussing the movement of nectar within the hive we use volume (μl), When calculating the concentration of a substance in the cell when a nectar load is added to it, the following equation is therefore used:

$$\text{Concentration in cell } [\mu\text{g} \cdot \text{l}^{-1}] = \frac{(\text{Concentration in cell } [\mu\text{g} \cdot \text{l}^{-1}] \cdot \text{volume of nectar in cell } [\mu\text{l}]) + (\text{Weight in nectar load } [\mu\text{g}])}{(\text{volume of nectar in cell } [\mu\text{l}] + \text{volume of nectar load } [\mu\text{l}])}$$

Process Overview and Scheduling

Time in the model is first split into days, at the beginning of the day, the 'daily update' procedure is called and at the end of each day nectar is processed. The main procedures of the model (Foraging, receiving storage and feeding) occur once per hour. Within these

procedures, when all agents perform an action (e.g. all receivers storing nectar) they are called at random to perform this action... Procedures are performed in the following order each day:

Daily update – Occurring at the start of each day, daily count variables are reset to 0. Larvae age, and if they are above the age threshold for pupation (by default 6 days), they are removed from the model as, in reality, they pupate and feeding ceases. Eggs are then laid in empty cells to replace the lost larvae, maintaining a constant number of larvae.

Foraging – Each hour while foraging time remains, a defined percentage of foragers are assigned, at random, to one of the two patches (treated with pesticide or non-treated). They are then given a set volume of nectar from the randomly assigned patch with the relevant sugar and pesticide concentrations.

Receiving – After each foraging round, receivers take the nectar loads from foragers, chosen randomly from the population of foragers still waiting to transfer nectar. After securing a nectar load the receiver chooses a cell in which to deposit nectar, depending on the scenario either at random or according to the sugar concentration of the nectar (clustering) and deposits the nectar load in the relevant cell.

Feeding – In the real world adult nurse bees feed the larvae, however as this is the only duty to be performed by nurse bees, in this model, nurse bees are implicit in the behavior

of the larvae, and the preparation of brood food is not modelled explicitly, as pollen is not included in this model. Feeding rates in the model do not depend on the source of the nectar, although in a real hive the sugar concentration of the nectar may lead to larvae being fed different volumes (Rortais *et al.*, 2005), the sugar concentration in this model is arbitrary, and by excluding this resultant differential volume used as food we do not limit ourselves to the scenario in which the pesticide is contained in nectar with a higher sugar concentration. Conversion from weight of nectar to volume of nectar would depend on the sugar concentration of the nectar. The sugar concentration of the nectar in this model is solely used as a label to differentiate between the two nectar sources, the fact that the treated nectar has a higher sugar concentration is arbitrary. It is therefore safe to assume the volume to weight ratio of 360 μ l of nectar to 500mg (0.72 μ l/mg) of nectar as used by Schmickl and Crailsheim (Schmickl and Crailsheim, 2007). This ratio is for honey in their model, however nothing is lost in this assumption for nectar in this model as feeding rates are not based on the sugar concentration. Every hour, the closest cell to each larva that contains enough nectar for one feed is chosen. The larvae then feed on the nectar from the relevant cell. Each hour, each larva receives 0.82 μ l nectar ($163.5 \cdot 0.72 \cdot 0.0069 = 0.82$), 163.5mg required to take one larva to pupation (Harbo, 2015), 0.72 – conversion to μ l, 0.0069 conversion to hours), assuming 6 days from hatching to pupation, with the conversion of mg to μ l as given above. In reality, the amount a larva is fed will change based on its age, as well as on the sugar concentration. We have kept the volume of nectar a larva eats constant across each day for simplicity. After the larvae have fed, the

foragers and receivers in the model feed, removing 0.32 μl per day (Rortais *et al.*, 2005). As nurse bees are only implicit they do not feed and their exposure is not considered.

Processing – Nectar cells which are more than 95% full are ‘capped’, so they are no longer available to be fed from or deposited in, and the nectar in them is concentrated, representing the transformation to honey. In the model, this processing is simply the reduction of the volume of the nectar by 75%, maintaining the weight of pesticide in the nectar constant (based on the simplified assumption that the nectar contains 80% water (Potts *et al.*, 2004), although in reality this is variable dependent on the species and climate, and that honey contains 20% water (Frankel, Robinson and Berenbaum, 2015)). As the sugar content of the capped nectar is of no consequence in this model and there is no repercussion on the exposure of the bees to the pesticide we consider this extreme simplification of the process is reasonable, acting as a placeholder for potential expansion of the model.

Design Concepts

Basic Principles

The basic principles of the model are those regarding the food storage, processing and feeding behaviors of the honeybee. The deposition of nectar in the comb has been reported,

or modelled in the past, as a random process(Montovan *et al.*, 2013)or by

following rules leading to a global pattern (Johnson, 2009). Yet others, have demonstrated that the concentration of sugar in the nectar could affect how nectar from different sources is

stored (Greco *et al.*, 2013). Multiple transfer of nectar from foragers to receivers has been

shown in numerous studies(Kirchner and Lindauer, 1994; Hart and Ratnieks, 2001), with 1.9 – 2.7 transfers being a representative number of transfers, and has the effect

of supplying increased information to foragers about the state of the colony(Hart and Ratnieks, 2001)while also potentially mixing nectar from different sources, increasing homogeneity of any pesticides being brought into the hive.

Emergence

The distribution of pesticide concentrations in the honey cells and in the larvae emerge from the foraging, storage and feeding procedures.

Adaptation

As nectar is brought into the hive and is stores receiver bees, if storing nectar in clusters, will ensure that they store nectar in or next to cells containing nectar of the same nectar concentration.

Sensing

Individuals are aware of the nectar quantity and quality (i.e. sugar concentration) in the honey cells on a global scale. They are also able to sense distance, allowing them to choose the nearest cell to store nectar in or take nectar from or to place nectar of similar concentrations together.

Interaction

There are three sources of interaction in the model: (1) between receivers and foragers when nectar is transferred, (2) between receivers and cells where nectar is deposited, and (3) between the larvae and the cells when the larvae feed.

Stochasticity

In one of the scenarios, the receiver bees place nectar at random in the comb as opposed to placing nectar near other nectar of similar sugar concentrations already in the comb.

The location of nectar in the comb upon initialization is stochastic. The precise layout of brood in the comb upon initialization is affected by randomized cell choices made by the queen.

Observation

The outputs from the model are the values for the pesticide amount in each larvae and pesticide concentration in each hive cells on each day. For each patch, due to nectar processing into honey, increasing the sugar and pesticide concentration as water is removed, pesticide amount per mg of sugar in the nectar/honey will also be recorded.

Initialization

At the beginning of the simulation, 150 foragers 150 receivers and 400 larvae are created. In a real brood frame, a much larger proportion of the cells could be filled with larvae during the breeding season, however a single side of a single frame is modelled here providing food for the larvae and adults. Larvae are placed in the comb so there are no more than two cells between each larva, similar to Johnson(Johnson, 2009). Initially 10% of the comb is filled with control (clean) nectar to represent that the frame has been used for brood and food storage for some time prior to a sudden pesticide-containing nectar flow. The concentration of pesticide in the nectar of the forage patch is set arbitrarily to 100 μg pesticide/L, intentionally high to ensure pesticide reaches the in-hive bees. The

model was created to test the extremes of the behaviors and not the precise movement of pesticide into the comb and will therefore not provide realistic values of pesticide in the individual bees. Instead an arbitrary value allows us to focus on how the different behaviors alter how the pesticide moves through the hive and the resulting heterogeneity of pesticide residues in nectar, adults and brood to evaluate which, if any of the extremes would be the worst-case scenario in terms of risk of exceeding a given toxicity threshold. The sugar concentration of the nectar acts purely as a label as to the source of the nectar, as there is some evidence that nectar could be clustered together based on sugar concentration (Greco *et al.*, 2013). This difference in sugar concentration between nectar from the two patches serves only to test receiver bee behavior; in reality the sugar concentration will be highly dependent on species and climate.

In this model, the pesticide does not dissipate and is not metabolized in the individual bees, e.g. during feeding of larvae. Dissipation and metabolism would be highly product specific and could greatly reduce the exposure of individuals to pesticide, by leaving it out from the model we ensure a conservative estimate of the exposure and maintain generality.

Input

The model does not rely on inputs from files or other models; the environment in the model is simple with just two food patches providing constant food.

Sub Models

There are four main procedures in the model, the foraging procedure, the receiving procedure, the processing procedure and the larval feeding procedure.

Foraging Procedure

All foragers are assigned to the control patch initially, and are given a nectar load with no sugar and the control sugar concentration. To simulate a treated patch being visited by a proportion of the foragers (default 0.25) that proportion of the foragers are chosen at random and their nectar variables are altered to represent the treated patch sugar concentration and pesticide amount. Real foraging in the honeybee colony is complicated and beyond the scope of this model if it was to be captured fully, this submodel is therefore simplified to the requirements of the model.

Receiving Procedure

After the foraging procedure, all the foragers are carrying a nectar load and queuing for the receiver bees. Each receiver bee then selects a forager from the population of foragers still carrying nectar. The receiver then takes the nectar from this forager, setting the sugar concentration and pesticide amount of its nectar load to that of the foragers. If multiple transfer is under investigation, two different foragers may be visited by the same receiver

Gregson et al 2003). The pesticide amounts from each forager are halved as it is assumed that both foragers transfer equal volumes. And the final nectar concentration is calculated as the sum of the individual concentrations each multiplied by half the volume of a nectar load divided by the volume of a nectar load. The receiver bees then store the nectar in the comb. If the receivers are set to act randomly, each receiver selects a cell at random, with enough room for a nectar load, to deposit their nectar load into, if they are acting non-randomly, they select a cell at random from all cells containing nectar with the same sugar concentration as their nectar load (\pm a range) and empty cells surrounding these. The receiver then moves to their chosen cell and add their nectar quantity to it and add their pesticide amount to the existing pesticide amount which is then divided by the new total quantity of nectar to give a concentration.

Processing Procedure

If nectar processing is enabled, when a nectar cell is more than 95% full, it will be processed to honey. Both the sugar concentration and the pesticide concentration will increase. Once the process is complete, the cell may be capped and removed from the possible cells for use by storing receivers or larvae, but counted in the measure of pesticide in the hive. Nectar is assumed to be 80% water (high estimate) and honey will

be defined as 20% water. Therefore as a conservative estimate we can assume that the nectar loses 75% of its volume.

Feeding procedure

Each larvae selects the closest cell containing enough nectar for one feed to itself. From Harbo et al 2003, it takes 163.5mg of nectar to raise a larvae to adulthood, and, assuming as in Schmickl and Crailsheim 2007 (Schmickl and Crailsheim, 2007), a cell can hold 500mg of nectar or 360µl, so it takes 117.72 of nectar to raise a larvae to adulthood. This value is divided by 6, the number of days a larvae exists in the model and then by 24 as the larvae are fed hourly, giving an hourly larval feeding amount of 0.82µl. The larvae takes an amount of pesticide equal to the pesticide concentration in the cell * 0.82 * 0.000001, to give the pesticide amount per larval hourly feeding amount in L.

Nectar is also removed from the comb to represent adult bee feeding. For each forager and receiver, each hour 0.33 µl are removed (calculated from a daily adult feeding amount of 11mg). As nurse bees are only implicit they do not feed and their exposure is not considered. This removal takes place randomly, with cells being chosen at random and nectar removed at random until enough removal has taken place.

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